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EXPERIMENTAL STUDIES IN CALCIFICATION

I. THE EFFECT OF A RACHITOGENIC DIET ON THE DENTAL TISSUES OF THE WHITE RAT *

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The purpose of this study was to investigate the effect of a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D upon the dental structures of the albino rat, and thus to establish a basis for subsequent experiments on the influence of parathyroid hormone, vitamin D, starvation, and injections of phosphate upon the dental tissues of the rachitic rat. This study was further indicated by conflicting findings reported in the literature regarding the occurrence of hypoplasia of the enamel in rickets.

REVIEW OF THE LITERATURE

While there are many reports on dental changes in experimental rickets (Erdheim,[1] Fleischmann,[2, 3] Gottlieb,[4] Bauer,[5] Mellanby,[6] and others), only investigations based on experimental diets comparable to the one used in this study, *e.g.*, diets high in calcium, low in phosphorus, and deficient in vitamin D, will be given primary consideration. Karshan and Rosebury [7] reported normal formation and calcification of the enamel and only slight and regional disturbances in the calcification of the dentin. They found an increased thickness of predentin only in the basal portion of the incisor. In the molars, wide predentin was confined chiefly to the roots and pulpal floor. Similarly, Lobeck [8] found in severe rickets only globular dentin and no changes in the enamel of the incisors. Becks and Ryder,[9] on the other hand, reported hypoplasia of the enamel in animals that had been on the rachitogenic diet for 4 weeks. Table I summarizes the data of the experimental studies on rickets.

* Received for publication, September 5, 1944.

Material and Methods

This study is based on material obtained from 33 white rats that were placed on a rachitogenic diet for a period of 1 to 56 days after weaning (at 21 days) (Table II). In addition, a study was made of

Table I

Data of Previous Experiments with Rachitogenic Diets Compared with Those of Present Investigation (High Calcium, Low Phosphorus, and Deficient in Vitamin D)

Author	Diet	Number of animals	Age at beginning	Duration of experiment	Histologic Findings		
					Alveolar bone	Incisor	
						Dentin	Enamel
			(*days*)	(*days*)			
Karshan and Rosebury[7]	Steenbock and Black No. 2965, modified by Epstein	?	33–41	38–57	Poorly calcified	Globulated predentin, apically	Not investigated
Lobeck[8]	McCollum No. 2638	12	0–28	18–126	Not investigated	Globulated	No changes
Becks and Ryder[9]	McCollum No. 3143	21	25	7–42	Poorly calcified	Poorly calcified	Hypoplasia
Weinmann and Schour	Steenbock and Black No. 2965 (modified)	33	21	1–56	Poorly calcified	Poorly calcified	No changes

25 litter-mates, 21 to 77 days of age, that were fed a normal control diet. The Steenbock-Black [10] diet no. 2965, modified by the substitution of corn meal for whole yellow corn, was used. The experimental animals gained less weight than did the controls.

The animals used in this and the subsequent studies were kindly made available by Drs. F. C. McLean and W. Bloom.

Table II

Summary of Histologic Findings in the Upper Jaw of 33 Rats on Rachitogenic Diet

Length of experiment	No. of rats	Incisor Average width*			Molar predentin	Cementoid		Cyst of enamel organ
		Predentin	Calcified dentin	Total dentin		Molar	Incisor	
(*days*)		(*microns*)	(*microns*)	(*microns*)				
1–4	7	15	111	126	Normal	Normal	Normal	None
5–6	6	18	112	130	Normal	Normal	Normal	+ (1)
7–8	3	23	97	120	Normal	Normal	Normal	None
21–56	17	64	70	134	2 to 3 times normal width	+ +	+ (13)	+ (8)

Numbers in parentheses indicate number of animals showing the disturbance.

* Measured at the level of completed enamel matrix (labial side).

*Histologic Preparation.** The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin. A number of ground sections of the incisors also were prepared.

Microscopic Findings

Upper Molars

Enamel. The enamel formed during the experimental period showed no significant changes.

Dentin. The predentin showed an increase in width, so that at the end of the experiment (after 56 days on the rachitogenic diet) the predentin reached a width of 50 to 60 μ, which is about three times that of the normal animal (Table II). The greatest width of predentin was found at the floor of the pulpal chamber. The width of predentin increased from the first to the third molar.

At the beginning of the experiment, approximately two-thirds of root formation was completed in the first molar. In the third molar, root formation had just begun. The second molar showed an intermediate stage of development. Those portions of the roots which had been formed during the experimental diet consisted almost entirely of predentin (Fig. 11). The pulp and odontoblasts showed no significant changes.

Cementum. The newly formed layer of cementum (precementum) did not calcify and was considerably wider than in the normal animal. The sum of the thickness of cementoid (or precementum) and calcified cementum was the same as in normal control animals, indicating a normal rate of deposition. Resorption of the cementum was much more frequent in the experimental animals (58 per cent) than in normal animals (12 per cent). The resorption areas were localized to the cervical half of the distal root surfaces. They were shallow but might extend into the dentin.

Upper Incisor

Enamel. The enamel of the incisor showed no disturbance in its formation, maturation, or pigmentation. Hypoplasia of the enamel in the sense of a lack of enamel structure was not observed.

The *enamel organ* was normal in the basal half of the tooth. In the

* Dr. H. Pitluck assisted in the technical part of this study.

incisal portion, however, cysts were found in about 50 per cent of the animals which had been on the experimental diet for 4 weeks. These cysts developed between the inner and outer enamel epithelium and probably originated by proliferation from the stratum intermedium (Fig. 1). They remained attached to the enamel surface in a restricted area and extended basally as a result of the eruptive movement of the tooth (Figs. 2 to 4). In some cases connective tissue had penetrated the degenerating lining of the cyst and filled the entire cavity (Fig. 4). In severe cases compression and degeneration of the enamel organ occurred as a consequence of the narrowing of the periodontal space.

Dentin. In the first week of the experiment the only effect was the appearance of a darkly stained incremental band in the enamel-covered dentin. This calciotraumatic line was seen 24 hours after the beginning of the experiment (Fig. 5), and shifted gradually away from the pulp in accordance with the continued apposition of dentin (Figs. 6 to 8). The line was more distinct in the middle and in the incisal third than in the basal third. The experimental dentin showed slightly less homogeneous calcification (interglobular dentin) than the pre-experimental dentin. This change was more pronounced also in the enamel-covered dentin than in the cementum-covered dentin.

Predentin. Beginning with the second week of the experiment, both the formation (Table II) and the calcification of the dentin were markedly retarded. The retardation of calcification was more pronounced than that of formation and as a consequence the width of the predentin in the basal third sometimes increased to four times the normal width (measured at the level of the completion of the enamel matrix) (Table II). The layer of predentin was thickest in the basal and narrower in the central and incisal portions. The zone of intermediate dentin (Schour and Rogoff [11]), which in normal animals was found in the middle third of the labial dentin, was absent in two-thirds of the animals after the third week of the experiment. The basal end of the dentin which was formed by the most recently differentiated odontoblasts tended to remain entirely uncalcified (Figs. 9 and 10, basal predentin) and varied in length from 0.35 mm. to 1.8 mm. It was always longer on the concave than on the convex side.

In 6 advanced cases the lingual basal predentin did not show a gradually increased width in the anterior direction but was abruptly demarcated from the more anterior and partly calcified dentin by a step in the pulpal outline (Fig. 10) as if the odontoblasts underwent a sudden spurt of increased activity at the time and place of initial calcification of dentin.

Vascular inclusions in the dentin were absent in animals that were on

the rachitogenic diet 3 weeks or less but were present in 6 of 15 rats that were on the rachitogenic diet for a longer period.

Differences in Reaction in Labial and Lingual Dentin. The differences in the behavior of the enamel-covered and the cementum-covered dentin were enhanced after the second week of the experiment. In the labial dentin the globules were large and remained separated from each other by interglobular dentin. In the lingual dentin the globules were small and irregular and the interglobular dentin was reduced to a minimum. It showed a characteristic striation.

Rate of Apposition. The formation of the dentin was appreciably retarded in experimental rickets so that the dentin was thinner than normal. The increase of the cross section of the tooth, however, which depends on epithelial and pulpal proliferation, continued at a normal rate. The result was a disharmony between the increase in the diameter of the tooth and increase in the thickness of its dentinal walls (Fig. 12, A and B).

Cementum. The calcified layer of the cementum of rachitic animals was very thin and of normal width. The cementoid layer, however, was thickened and contained some cells (Table II). The evenly spaced dental fibers of the periodental membrane were anchored in the cementoid as Sharpey's fibers and gave it a striated appearance.

Discussion

Differences in the Reactivity of the Calcified Structures. The disturbances of the calcified dental tissues in experimental rickets were confined to the dentin and cementum. The formation of predentin was retarded and the calcification of the cementum and the dentin was impaired. When a part of the predentin formed during the experiment calcified, the calcification was incomplete, as shown by the persistence of large areas of interglobular dentin. The layer of predentin increased in width. The calcification of the newly formed cementum either was lacking or considerably restricted.

The enamel seems to be entirely immune to the metabolic disturbances of the rickets produced by our experiment. Not only is the formation of enamel undisturbed, but the maturation (calcification) of the enamel proceeds normally. The difference in the behavior of the enamel from that of the other hard tissues (dentin, cementum, and bone) may be caused by the chemical difference of the matrix of these different tissues. No principal differences exist in this respect between bone, cementum, and dentin, all mesodermal tissues. The enamel matrix, however, as an epithelial product, shows tinctorial peculiarities, at least, which indicate a different chemical composition.

The rachitic changes in bones are more complex because, in addition to the disturbance in calcification, there are the secondary changes which are caused by the resistance of uncalcified cartilage and bone to resorption and which are entirely lacking in the dental tissues. Resorption constitutes an essential phase in the development and growth of bones but plays no part in the development and growth of the incisors and molars of the rat.

Retardation in the Calcification of the Dentin. In rickets the predentin is significantly wider in the apical third but tends to be approximately of normal width in the middle and incisal thirds.

Since, with the continuous eruption of the incisor, the basal third of the dentin eventually moves anteriorly into the middle portion of the tooth, and since in that portion of the tooth calcification tends to be normal, the disturbance in calcification must be one of retardation in the rate of calcification. The retardation in the rate of calcification of dentin is further indicated by the fact that the predentin is not only wider, but persists longer, than normally, especially at the lingual side (basal predentin).

Hypoplasia of Enamel. The absence of hypoplasia of the enamel in our material confirms the findings of Karshan and Rosebury [7] and of Lobeck.[8] The cystic degeneration of the enamel organ was confined to the incisal half of the incisor where the enamel had already been completed in its formation and calcification, and therefore should not be considered as an enamel defect (Gottlieb [4]).

Several authors [12-15] have found hypoplasia of the enamel in 20 to 25 per cent of cases of rickets, while Fleischmann,[2, 3] whose findings were based on histologic and post-mortem evidence, found no relationship between hypoplasia of the enamel and rickets.

It seems that rickets *per se,* which results from a diet high in calcium, low in phosphorus, and deficient in vitamin D, is not responsible for hypoplasia of the enamel.

Summary and Conclusions

This investigation was based on a study of the teeth of 33 white rats which were placed after weaning on a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D for a period of from 1 to 56 days, and of 25 litter-mate controls.

The histologic findings were:

1. Enamel formation and calcification were normal. Hypoplasia of the enamel was absent. The enamel organ showed cystic degeneration in the incisal half of the incisor.

2. Dentin formation was retarded in rate. Calcification of dentin was

retarded and also disturbed. A calciotraumatic line was the immediate response which marked the beginning of the experiment. The newly formed dentin showed an interglobular texture. The enamel-covered dentin was more severely affected than the cementum-covered dentin.

3. Cementum formation was normal in rate but its calcification was defective. Shallow areas of resorption on the molar roots were more frequent than in normal animals.

REFERENCES

1. Erdheim, J. Rachitis und Epithelkörperchen. *Denkschr. d. k. Acad. d. Wissensch., Math.-naturw. Klasse, Wien,* 1914, **90,** 363–683.
2. Fleischmann, L. Die Ursache der Schmelzhypoplasien. *Vrtljsschr. f. Zahnh.,* 1909, **25,** 868–905.
3. Fleischmann, L. Rhachitische Veränderungen des Dentins. *Vrtljsschr. f. Zahnh.,* 1910, **26,** 11–21.
4. Gottlieb, B. Rachitis and enamel hypoplasia. *Dental Cosmos,* 1920, **62,** 1209–1316.
5. Bauer, W. Zur Entstehung der rachitischen Schmelzhypoplasien. *Vrtljsschr. f. Zahnh.,* 1929, **45,** 62–79.
6. Mellanby, M. Diet and the teeth: an experimental study. Part I. Dental structures in dogs. *Medical Research Council, Special Report Series, No. 140,* His Majesty's Stationery Office, London, 1929.
7. Karshan, M., and Rosebury, T. Correlation of chemical and pathological changes in teeth and bones on rachitic and non-rachitic diets. *J. Dent. Research,* 1933, **13,** 305–310.
8. Lobeck, E. Über experimentelle Rachitis an Ratten. *Frankfurt. Ztschr. f. Path.,* 1924, **30,** 402–442.
9. Becks, H., and Ryder, W. B. Experimental rickets and calcification of dentin. *Arch. Path.,* 1931, **12,** 358–386.
10. Steenbock, H., and Black, A. Fat soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. *J. Biol. Chem.,* 1925, **64,** 263–298.
11. Schour, I., and Rogoff, J. M. Changes in the rat incisor following bilateral adrenalectomy. *Am. J. Physiol.,* 1936, **115,** 334–344.
12. Dick, J. L. Rickets. W. Heinemann, London, 1922, p. 202.
13. Eliot, M. M., Souther, S. P., Anderson, B. G., and Arnim, S. S. A study of the teeth of a group of school children previously examined for rickets. *Am. J. Dis. Child.,* 1933, **46,** 458–461; *Ibid.,* 1934, **48,** 713–729.
14. Mackay, H. M. M., and Rose, S. F. Vitamin D deficiency, dental caries, and tonsillar enlargement. *Lancet,* 1931, **2,** 1230–1235.
15. Sarnat, B. G., and Schour, I. Enamel hypoplasia (chronologic enamel aplasia) in relation to systemic disease: a chronologic, morphologic, and etiologic classification. *J. Am. Dent. A.,* 1941, **28,** 1989–2000; *Ibid.,* 1942, **29,** 67–75.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 135

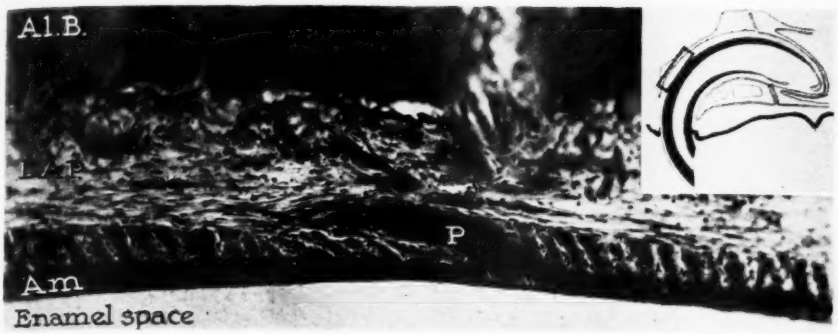
Photomicrographs of decalcified, longitudinal, midsagittal sections of the labial anterior region of the upper incisor of rats weaned and placed on a rachitogenic diet at 21 days of age. The area is indicated in the insert in Figure 1. Al.B. = labial alveolar bone; Am. = ameloblasts. The space next to the ameloblasts was originally occupied by enamel which is lost during decalcification. All illustrations were prepared from sections stained with hematoxylin and eosin.

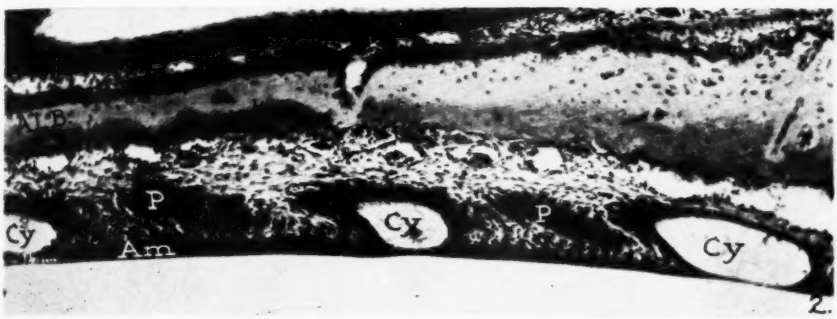
FIG. 1. Rat 404, 50 days old. Section shows solid proliferation (P) of the stratum intermedium and outer enamel epithelium and the normal smooth surface of the ameloblastic layer. L.A.P. = labial alveolar periosteum. × 119.

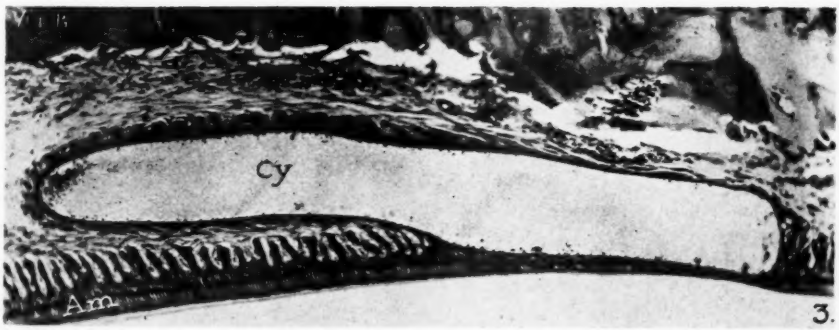
FIG. 2. Rat 1602, 52 days old. Multiple sites of proliferation (P) and cysts (Cy) of the stratum intermedium and outer enamel epithelium are indicated. × 88.

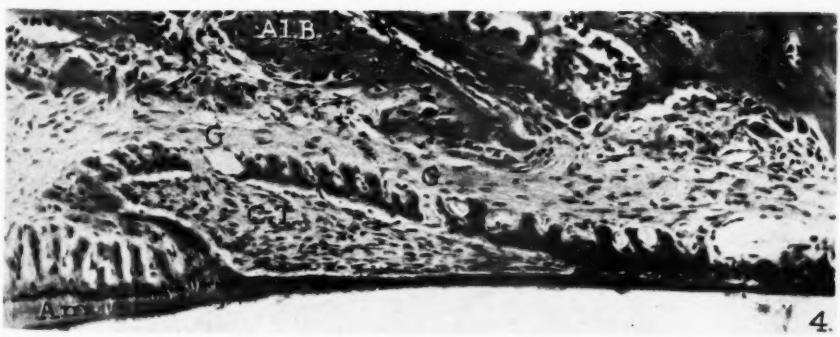
FIG. 3. Rat 1501, 49 days old. A single large cyst (Cy) has its wall formed by the stratum intermedium and the partly degenerated outer enamel epithelium. × 88.

FIG. 4. Rat 303, 54 days old. A large cyst is filled with connective tissue (C.T.), which has apparently penetrated through gaps (G) in the epithelium. The surface of the enamel is smooth. × 119.









Weinmann and Schour Effect of Rachitogenic Diet on Dental Tissues

PLATE 136

FIGS. 5–8. Photomicrographs of longitudinal sections of the labial dentinal wall from the middle third of the upper incisor. A dark-staining stripe in the dentin, the calciotraumatic line (X), represents the immediate response to the rachitogenic diet and marks the beginning of the experiment. × 102.

FIG. 5. Rat 3203, 22 days old, placed on rachitogenic diet for 24 hours. The calciotraumatic line (X) is next to the predentin.

FIG. 6. Rat 3205, 25 days old, placed on rachitogenic diet for 3 days. The calciotraumatic line (X) is 47 μ distant from the odontoblastic layer.

FIG. 7. Rat 3207, 27 days old, placed on rachitogenic diet for 5 days. The calciotraumatic line (X) is 84 μ distant from the odontoblastic layer.

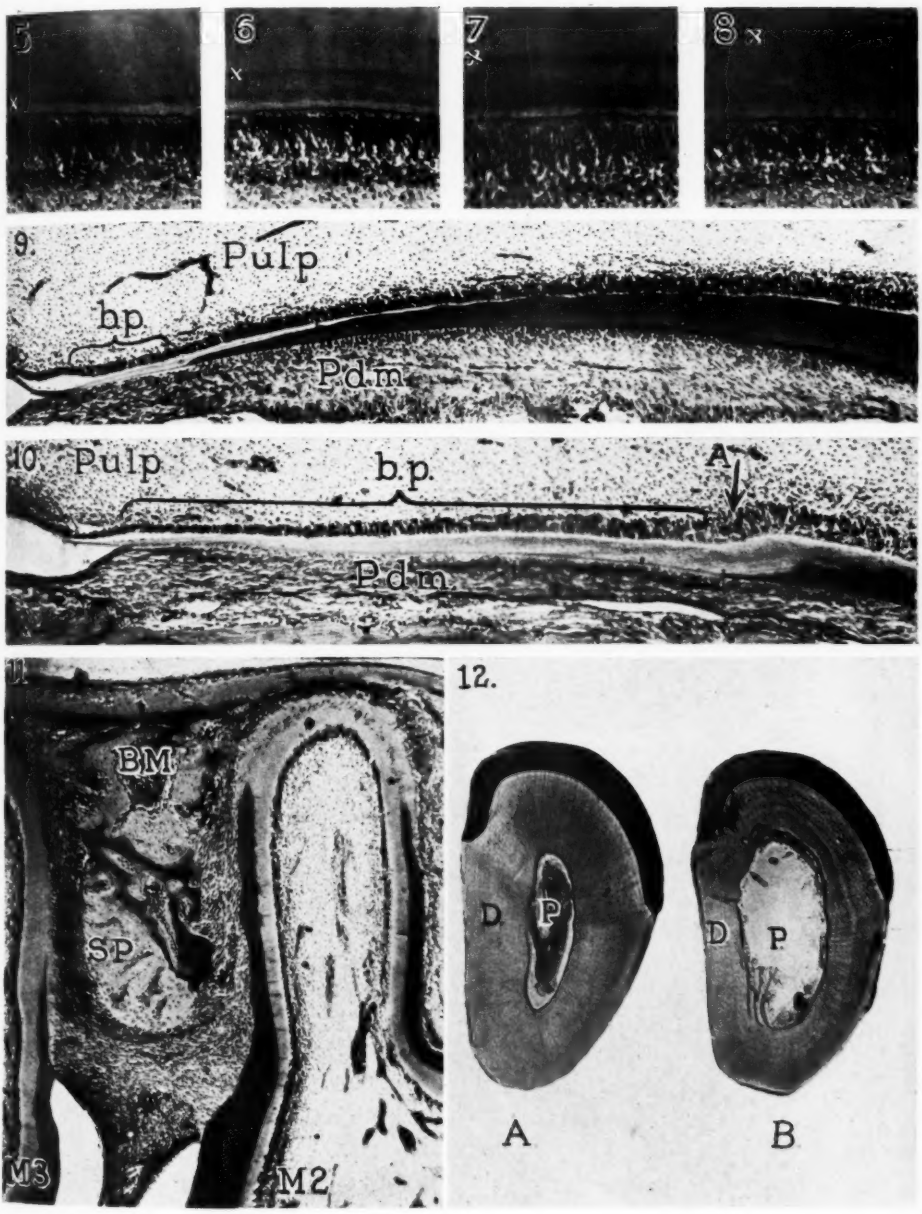
FIG. 8. Rat 3209, 29 days old, placed on rachitogenic diet for 7 days. The calciotraumatic line (X) is 112 μ from the odontoblastic layer.

FIG. 9. Photomicrograph of a longitudinal section of the lingual basal portion of the upper incisor of rat 1801, which was 67 days of age and was placed on the control basal diet after weaning at 21 days of age. Of note are the short uncalcified basal area, the basal predentin, b.p., and the smooth pulpal surface. P.d.m. = periodontal membrane. × 53.

FIG. 10. Photomicrograph of area corresponding to that of Figure 9 taken from rat 1802, which was 67 days of age and was fed the rachitogenic diet for 46 days. Here are seen the extensive uncalcified basal area (b.p.) and the sudden increase in dentin thickness at A. × 53.

FIG. 11. Photomicrograph of mesiodistal section of distal root of second (M_2) and mesial root of third (M_3) upper molar of rat 1902, 49 days of age, which was weaned at 21 days of age and then placed on a rachitogenic diet for 28 days. Section shows the difference in the calcification of dentin and cementum in the roots of the second and third molar, reduction of the bone marrow spaces (BM), and the eccentric location of the pre-experimental calcified core in the interdental alveolar septum (SP). × 58.

FIG. 12. Photomicrographs of transverse ground sections of the incisor of normal rat 1801 (A) and rachitic rat 1802 (B). The animals were litter-mates, 67 days old. The circumference of the teeth is essentially the same but the dentin (D) of the normal rat (A) is wider and the pulpal cavity (P) narrower than in the rachitic animal (B). × 58.



Weinmann and Schour Effect of Rachitogenic Diet on Dental Tissues

UNIV. OF MICH.

EXPERIMENTAL STUDIES IN CALCIFICATION

II. The Effect of a Rachitogenic Diet on the Alveolar Bone of the White Rat *

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The studies of bone growth in rickets thus far reported have been concerned chiefly with disturbances in growth of endochondral bone. Reports on the rachitic disturbances in endomembranous bone, uncomplicated by the presence of growing cartilage, are lacking except for frequent statements that the bone trabeculae are characteristically bordered by osteoid (Erdheim,[1] Becks and Ryder,[2] McLean [3]). Similarly, little attention has been given to a consideration of the resorption pattern in rachitic bone. The alveolar bone of the incisor and molar regions of the albino rat may serve as a good test object in the analysis of both apposition and resorption, since this bone is undergoing relatively rapid and continuous architectural reconstruction coincident with the physiologic movement of the teeth. In addition, the bone is always in a relatively constant relationship to the tooth which serves as a ready point of reference. An analysis of the alveolar bone, therefore, promises to be of special significance for the understanding of rachitic bone changes in general.

Material and Methods

The material and methods were the same as those used for the preceding paper (Table I).[4] Thirty-three white rats † were placed on a rachitogenic diet for a period of 1 to 56 days after weaning (at 21 days). In addition, a study was made of 25 litter-mates, 21 to 77 days of age, fed on a normal control diet. The Steenbock-Black [15] diet no. 2965, modified by the substitution of corn meal for whole yellow corn, was used.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and portions bearing the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The sections were stained with hematoxylin and eosin.

Microscopic Findings

A description of the findings in the experimental animals will be preceded by a brief presentation of the normal histologic structures.

* Received for publication, September 5, 1944.

† The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

A. UPPER MOLAR REGION

Normal Animals

The alveolar bone (Text-Fig. 1-A and Fig. 1), as seen in mesiodistal section of the upper molar, consists of (1) a thin, compact basal plate which forms part of the boundary of the temporal fossa and part of the alveolar wall of the apices of the roots, and (2) the spongy interdental and interradicular septa. The periodontal borders of the septa tend to be continuous. The septum between the first and second molars assumes a triangular form. Its gingival half is dense and contains small marrow spaces that are filled with loose connective tissue. Its basal half contains larger intercommunicating spaces that are filled with red marrow.

The alveolar process in the bifurcation of the roots presents a spongy framework of thin bone trabeculae. It consists of a large central portion which is filled with red marrow, and a relatively narrow peripheral portion. The latter borders the periodontal membrane and contains loose connective tissue in its marrow spaces.

The osteoblasts are numerous at the crest of the interradicular and interdental septa. They are less prominent in the small bone-marrow spaces near the periodontal membrane. Osteoclastic resorption is seen frequently at the endosteal surfaces of the bone trabeculae.

Corresponding to the distal drift of the molars, bone apposition can be seen on the mesial alveolar walls, bone resorption on the distal walls. An exception is the alveolus of the mesial root of the first molar. The divergence of this root from the other roots of the first molar necessitates resorption at the mesial wall during the tipping occluso-distal movement of this tooth. The drifting movement of the rat molars continues throughout the life of the animal. It is, however, intermittent. During the rest periods thin layers of bone are apposed in small restricted areas upon the resorbed bone and the principal fibers of the periodontal membrane are reattached to the alveolar wall (Sicher and Weinmann [5]).

The bone trabeculae stain intensively with hematoxylin, suggesting normal calcification. Osteoid borders are almost entirely missing. Only an occasional extremely delicate eosin-staining seam is found at the crest of the interdental and the interradicular septa.

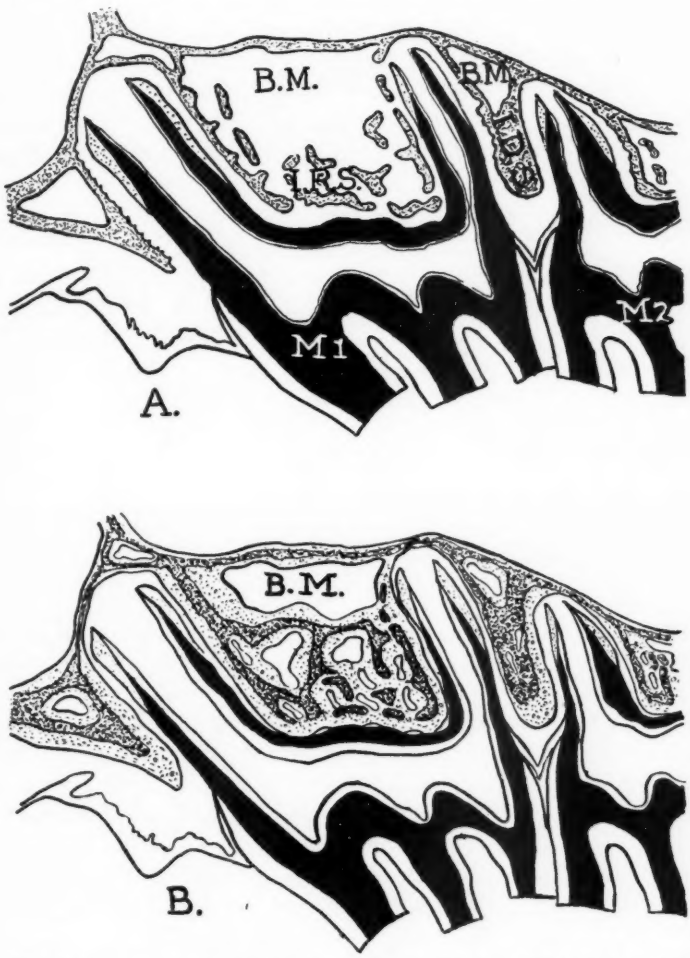
The *periodontal membrane* is of fairly uniform width.

Experimental Animals

Alveolar Bone. The alveolar bone presented a normal picture (Fig. 1) until the fifth day on the rachitogenic diet. At this time the alveolar crest showed an osteoid seam which (Table I) continued to increase in thickness throughout the duration of the experiment (Text-

Fig. 1 and Fig. 2). The maximal width of the osteoid tissue was not greater than the expected apposition of bone in a given area (Hoffman and Schour [6]).

As a result of the distal movement of the molars (Sicher and Wein-



Text-Fig. 1. *A*. Semidiagrammatic tracing of the first (M_1) and mesial half of the second (M_2) upper molar of a normal rat. The interradicular septum (I.R.S.) consists of thin, well calcified trabeculae enclosing a large marrow cavity (B.M.). The interdental septum (I.D.S.) is formed by a thick lamina at the crest, and trabeculae at the base enveloping a large marrow space (B.M.). The periodontal membrane, which is located between the alveolar bone and the roots, is of fairly even width. *B*. Semidiagrammatic tracing of same region as shown in *A*, but taken from a rachitic rat. The trabeculae are increased in thickness and the marrow spaces (B.M.) are decreased in size by apposition of osteoid tissue (lighter stippled areas). The periodontal membrane is reduced in width and for the most part obliterated at the crest of the interradicular septum.

mann [5]) the mesial alveolar wall showed apposition of osteoid tissue which gradually increased in thickness. The distal alveolar walls, on the other hand, showed chiefly progressive resorption, apposition of osteoid tissue occurring only in a few restricted areas. In the area of the first molar, because of the divergence of its roots, apposition of osteoid tissue was found at the distal, and resorption at the mesial, alveolar wall of the mesial root.

The trabeculae of the spongy bone in the alveolar septa were covered also by a layer of osteoid tissue which increased in thickness with the duration of the experiment. This proceeded at the expense of the bone-

TABLE I

Summary of Histologic Findings in the Upper Molars, Incisors, and Related Supporting Tissues of 33 Rats on a Rachitogenic Diet

Length of experiment	No. of rats	Osteoid alveolar bone			Ankylosis of periodontium of molar	Reduction of labial alveolar periosteum
		Molar	Incisor			
			Ling. plate	Lab. plate		
(*days*)						
1–4	7	N	N	+	N	N
5–6	6	?	N	+	N	+ (3)
7–8	3	+	+	+	N	+ (3)
21–56	17	+++	+++	+++	+ (10)	+ (4) ++ (11)

Numbers in parentheses indicate number of animals showing the disturbance.
N = No recognizable deviation from normal.
+ = Recognizable deviation from normal structure or the presence of the abnormality when specifically indicated.

marrow spaces (Fig. 2). Osteoclastic resorption, which was characteristic in the control animals, was almost entirely absent in the osteoid-covered trabeculae.

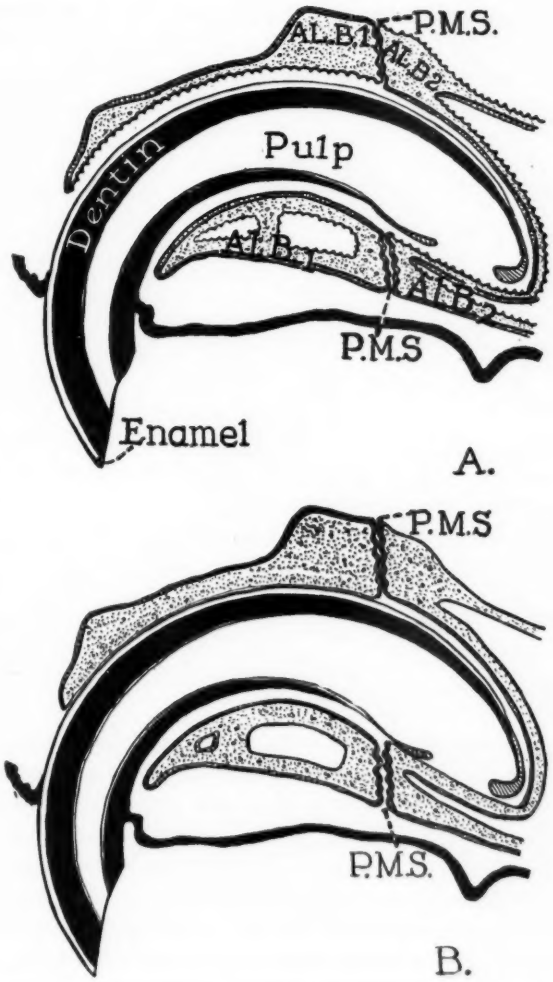
Periodontal Membrane. The first changes in the periodontal membrane occurred after the third week of the experiment. The periodontal space was narrowed and the periodontal tissues were compressed at the sites of physiologic bone apposition. The changes were most severe in the region of bifurcation at the height of the interradicular septa. Complete obliteration of the periodontal space (Fig. 2) occurred in this area in 10 of 17 cases (Table I). At the periapical region the width of the periodontal membrane remained normal.

B. UPPER INCISOR REGION

Normal Animals

The alveolar bone of the upper incisor is divided into an anterior premaxillary and a posterior maxillary portion by the premaxilla-maxillary

suture (Text-Fig. 2-A). The suture runs in an almost vertical plane. The anterior portion constitutes the anterior two-thirds of the labial bone and almost the entire lingual alveolar bone. The posterior portion



Text-Fig. 2. *A*. Semidiagrammatic tracing of the upper incisor of a normal rat. The alveolar bone consists of the anterior (Al.B. 1) and posterior portions (Al.B. 2) which are separated by the premaxilla-maxillary suture (P.M.S.). Scalloped borders indicate sites of resorption; double bars with crossbars indicate sites of apposition (see Table II). *B*. Semidiagrammatic tracing of the upper incisor of a rachitic rat, showing absence of sites of resorption. The width of the periodontal membrane is reduced or the membrane partially absent in regions where resorption normally occurs (see Table II).

constitutes the fundus and the posterior third of the labial alveolar bone. The labial alveolar bone consists of a thin premaxillary plate and a maxillary portion which serves as a thimble in which the basal end of the incisor rests.

The lingual alveolar bone (Text-Fig. 2 and Fig. 3) is sickle-shaped in longitudinal section. It consists of a periodontal plate, which parallels the curvature of the concave surface of the incisor, and a somewhat thicker periosteal plate which parallels and faces the palate. Both

TABLE II

Sites of Apposition and Resorption in Alveolar Bone of Upper Incisors of Normal Rat and Sites of Apposition in Rickets

Divisions of alveolar bone	Subdivisions		Normal		Rickets*
			Sites of		
			Apposition	Resorption	Sites of Apposition
Anterior or premaxillary portion of alveolar bone	Anterior two-thirds of labial alveolar bone	Periosteal	+		+
		Alveolar		+	−
	Lingual alveolar bone	Periodontal	+		+
		Endosteal		+	−
		Palatal	+		+
Posterior or maxillary portion of alveolar bone	Posterior third of labial alveolar bone	Alveolar	+		+
		Nasal		+	−
	Fundus	Nasal	+		+
		Alveolar		+	−

* Resorption is inhibited in rickets.

plates converge incisally at an acute angle at the lingual alveolar crest and are joined by a few transverse trabeculae. The lingual alveolar bone contains in its center spaces filled with islands of red marrow that are embedded in fatty marrow. A blind recess of the maxillary sinus extends into the posterior portion of this bone. The presence of an extremely thin osteoid border at the periodontal surface indicates relatively fast apposition of bone at this site. Howship's lacunae and osteoclasts are present occasionally along the endosteal surfaces.

The fundic bone is extremely thin. It is bordered on its nasal surface by a layer of mucous glands of the maxillary sinus (Fig. 10).

Table II gives the normal sites of apposition and of resorption in the alveolar bone of the upper incisor region.

Periodontal Membrane. The periodontal membrane, as a specialized connective tissue, may be classified into three portions:

1. The Periodontal Membrane Proper. The periodontal membrane

proper is confined to the concave and lateral cementum-covered surfaces of the incisor and serves as a suspensory ligament. In adaptation to the continuous eruption of the tooth, this ligament is arranged in three distinct layers (Fig. 5): the alveolar fibers, the dental fibers, and the intermediate plexus. The alveolar and dental fibers are arranged in a radial direction, *i.e.*, at right angles to the surface of the tooth. The alveolar fibers form strong bundles between which vessels and nerves are contained in loose connective tissue. The vessels are near the bone surface and run at right angles to the long axis of the tooth. The dental fibers form an almost continuous layer.

2. The Labial Alveolar Periosteum. The labial alveolar periosteum is confined to the convex side of the tooth (Fig. 7). It lies between the enamel organ and bone and consists of dense connective tissue, the fibers of which run parallel to the surface of the bone. The tissue is highly vascularized, with most of the vessels running close to the surface of the bone and parallel to the long axis of the tooth. Corresponding to the bones which form the alveolus at the convex side of the tooth, the labial alveolar periosteum consists of a premaxillary and maxillary portion.

3. The Fundic Portion. The fundic portion lies between an epithelial diaphragm and the fundic bone and is differentiated into the hammock ligament upon which the basal ends of the incisors rest and into a layer of very loose connective tissue which is adjacent to the tooth (Fig. 10).

Experimental Findings

Alveolar Bone. The reaction of the alveolar bone was different in the normal sites of bone apposition and in the normal sites of bone resorption.

In the normal sites of bone apposition the new formation of bone proceeded at a fairly normal rate but the calcification of the newly formed matrix was lacking. Here an increase in the width of osteoid tissue was observed beginning with the second week of the experiment (Text-Fig. 2 and Table II). Apposition of osteoid tissue (Table I) also occurred along the trabeculae of the spongy bone, especially in the lingual alveolar plate (Figs. 3 and 4). The gradual thickening of the trabeculae led to a narrowing of the marrow spaces.

In the normal sites of bone resorption (Text-Fig. 2) resorption proceeded until the calcified bone had been removed and the remaining bone consisted entirely of osteoid tissue when resorption no longer occurred. The bone now gradually increased in thickness because the continued apposition was not balanced by simultaneous resorption (Figs. 7 to 11).

Immediately anterior to the premaxilla-maxillary suture (Text-Fig.

2) the thin labial alveolar plate was connected with the frontal process of the premaxilla by a number of vertical bone trabeculae. These trabeculae were normally covered by osteoblasts. During the fifth week of the experiment they showed in many places wide seams of osteoid tissue, whereas their core consisted of well calcified bone (Fig. 12). When the resorption, proceeding at the periodontal surface of the premaxilla, reached this system of trabeculae, the different reaction of osteoid tissue and bone could be observed. Osteoclasts were seen attacking the calcified core of the trabeculae, whereas the osteoid tissue did not show signs of osteoclastic resorption (Fig. 13).

The Periodontal Membrane. The periodontal membrane proper, at the lingual or cementum-covered side of the tooth, remained, as a rule, normal in width. A narrowing of the space was observed only once. In advanced cases the arrangement of the suspensory fibers was markedly disturbed. The normal arrangement of the periodontal fibers (Fig. 5) in three layers was almost lost in the incisal portion where they followed a direct course from bone to the cementum (Fig. 6). A number of lymphoid wandering cells were found between the bundles of the principal fibers.

The labial alveolar periosteum of the tooth showed a marked difference in the premaxillary and maxillary portions of the alveolus. In the maxillary part no significant changes were seen. In the premaxillary part a gradual narrowing of the periodontal space (Table I) led to an increasing compression of the connective tissue and blood vessels (Figs. 8 and 9). In advanced stages the connective tissue underwent hyaline degeneration. The hyaline changes appeared in isolated areas as round or oval islands which later fused to form a trabeculae-like network (Figs. 8 and 9). The latter included connective tissue cells and sometimes multinucleated giant cells (osteoclasts). In its staining reaction the hyalin resembled osteoid tissue. Differentiation of the hyalin and the adjacent osteoid tissue was not always sharp. The absence of osteoblasts indicated that this tissue was not the result of apposition.

The fundic connective tissue underwent changes very similar to those of the premaxillary alveolar periosteum. The fundic space was gradually narrowed and the loose connective tissue and its blood vessels were compressed. In advanced stages the tissue adjacent to the bone underwent hyaline degeneration (Fig. 11).

Discussion

Growth of bone and its maintenance as a functioning organ is dependent upon the balanced interaction of bone apposition and bone resorption. The processes of apposition and resorption have to be

studied with equal emphasis in order to understand fully the rachitic changes of the skeleton. The alveolar bone of the rat is a useful test object for such studies. The normal relations between bone and tooth and between the growth changes of the bone and the growth changes and physiologic movement of the teeth have been sufficiently well clarified to form a basis for experimental analysis (Sicher and Weinmann [5]). The pathologic changes in alveolar bone can be studied by examining the disturbances of the relation between bone and tooth. A rachitic diet acts primarily on the bone and dentin, but does not influence the shape of the teeth (Weinmann and Schour [4]).

Our findings agree with the generally accepted view that the primary cause of the pathologic changes following rachitogenic diet is the failure in calcification (McLean,[3] Pommer,[7] Howland,[8] Dodds and Cameron [9]). The mechanism by which some of the rachitic deformities are produced is secondary and depends on the peculiar anatomy and physiology of each region, and especially on the local pattern of apposition and resorption.

A. The Spongy Bone

In the spongy bone next to the bone marrow the trabeculae thicken and the marrow spaces become narrow as a result of the slackening and final cessation of resorption. The reason for the inhibition of resorption appears to be the failure of the newly formed bone to calcify. Osteoid tissue is almost immune to resorption, as are the uncalcified cementum (cementoid) and the uncalcified dentin (predentin) (Gottlieb,[10] Orbán and Weinmann,[11] Kronfeld [12]). The resistance of osteoid tissue to resorption seems to be caused not only by its lack of calcium salts but by the lack of those changes in the organic matrix which precede or accompany calcification. That the rachitic changes in spongy bone are a result of an inhibition of resorption and not of an overproduction of new bone is indicated by the fact that osteoclasts are absent in advanced stages and that measurements at sites of regular undisturbed bone formation, as at the crest of the alveolar septa of the molars, indicate a normal rate of bone apposition. McCollum, Simmonds, Shipley, and Park [13] observed a reduction in resorption in experimental rickets. They explained this lack of resorption on the basis of changes occurring in the osteoblasts and cartilage cells.

B. The Alveolar Bone of the Molar Region

In a majority of cases an encroachment of the periodontal membrane in the region of bifurcation occurred after 3 weeks on the experimental diet. A reduction in width of the periodontal membrane was found on

the mesial side, whereas the periodontal membrane remained normal on the distal side and at the fundus. These findings may be explained on the basis of the normal growth pattern of the jaws. The occlusal movement of the molars is coordinated with, and made possible by, the increase of distance between maxillary and mandibular body as a consequence of the growth of the mandibular ramus. The latter increases in height by endochondral bone growth at the mandibular condyle. The condylar growth is in every respect the same as the longitudinal growth of the shaft of the long bone at the epiphyseal cartilage.

We know that the endochondral epiphyseal growth of bone is considerably retarded in experimental rickets (Dodds and Cameron [9]), and probably the same holds true for the condylar growth of the mandible. Thus space for the eruption of the molars cannot be provided to the normal extent in rachitic rats and the occlusal movement of the molars is therefore mechanically checked.

In the normal rat the alveolar bone of the molar shows three distinct zones of apposition (Hoffman and Schour [6]). The greatest amount of apposition occurs at the crest of the alveolar septa, less at the mesial alveolar, and least at the fundus. It has been suggested (Sicher and Weinmann [5]) that the apposition at the crest of the interradicular septa causes the vertical eruption of the molar, that the apposition of bone at the fundus is secondary to the occlusal eruption, and that the distal drift of the rat molar is caused by apposition of bone at the mesial alveolar wall. As the apposition of bone at the crest of the interradicular septum of rachitic animals proceeds, the periodontal spaces at the bifurcation of the immobilized molar is gradually narrowed and finally obliterated. Ankylosis between bone and molars in rachitic rats was reported previously (Becks and Ryder,[2] Orbán and Weinmann [11]). The progressive apposition of bone at the mesial alveolar wall narrows the mesial periodontal space of the immobilized tooth. The bone formation at the fundus of the molar is greatly inhibited, apparently because the tooth is not elevated in its socket.

C. The Alveolar Bone of the Upper Incisor Region

A marked reduction of the periodontal membrane has been observed along that part of the alveolus where resorption normally occurs, that is, the premaxillary labial portion and the maxillary fundic portion (Figs. 9 and 11). Since the reason for this physiologic resorption is different in these two areas, the mechanism of the pathologic changes will be considered separately.

The Premaxillary Labial Portion. In the premaxilla the alveolus of the normal rat is widened by resorption at the convex side of the tooth,

mainly because of the increase of the diameter of the incisor (Sicher and Weinmann [5]).

The widening of the alveolus in the premaxilla comes to a standstill in rachitic rats when the calcified bone present at the beginning of the experiment has been resorbed. Then the premaxillary alveolar plate at the convex side of the tooth consists only of osteoid tissue, which is resistant to resorption. Since the growth of the incisor in diameter progresses normally (Weinmann and Schour [4]), the tooth encroaches upon the periodontal tissues on the convex side (Figs. 8 and 9). Thus the width of the periodontal space is narrowed and later on the periodontal tissues are compressed between the growing tooth and the unyielding osteoid tissue. The impairment of the circulation of the blood in this area leads to hyaline degeneration of the connective tissue. The premaxillary periodontal space on the convex side of the incisor is finally obliterated.

The Maxillary Fundic Portion. Sutural growth between maxilla and premaxilla complicates the relation between the maxillary part of the alveolus and the teeth. If the tooth is considered as the fixed point, growth in the premaxilla-maxillary suture moves the fundic plate toward the basal end of the tooth and the maxillary alveolar plate at the convex side of the tooth away from the tooth. A reduction of the periodontal width at the fundus and an increase in the periodontal width at the convex side of the tooth would result if these tendencies were not compensated by resorption at the fundus and apposition at the maxillary alveolus on the convex side of the tooth (Text-Fig. 2). This picture of the normal animal has to be taken as a basis for the understanding of rachitic changes. In advanced rickets, the resorption of osteoid tissue fails and therefore the resorption of the fundic bone ceases. Since, however, growth in the suture continues, the periodontal width continues to be reduced (Fig. 11) and the periapical tissue may even become compressed and may undergo hyaline degeneration. The widening of the maxillary periodontal space at the convex side of the incisor by sutural growth and the apposition of osteoid tissue in those areas continue. The maxillary part of the periodontal membrane at the convex side of the tooth shows, therefore, no pathologic changes in rachitic rats.

Eruption

The eruption of the teeth in experimental rickets is retarded. Although no actual measurements of the rate of eruption have been made in rachitic animals, the histologic observations are convincing. The encroachment of the periodontal membrane between interradicular septa and the molars indicates clearly an arrest of the vertical movement of

these teeth. The retardation of the eruption of the rat incisor is indicated by the formation of a thick layer of cementoid tissue and by the disorganization of the periodontal membrane (Fig. 6). The division of periodontal membrane into three layers, which is an adaptation to rapid longitudinal eruption (Sicher [14]), disappears almost entirely. As discussed previously, the disturbance of eruption appears to be secondary to the retardation of bone growth at the mandibular condyle.

Summary and Conclusions

This histologic study is based on 33 rats which were placed on a rachitogenic diet for a period of 1 to 56 days beginning with weaning. The histologic findings were:

1. The formation of new bone seems to proceed at a normal rate but it remains uncalcified and persists as osteoid tissue.
2. Osteoid tissue fails to undergo resorption.
3. The consequences of the failure of resorption of osteoid tissue are:

a. Excessive accumulation of osteoid tissue. The sites which normally show apposition continue to do so while the sites which normally show resorption are inactive.

b. Distortion of the growth pattern of the alveolar bone, since the normal growth pattern depends upon a balance between apposition and resorption.

c. Reduction of the periodontal space in definite areas. Compression of the periodontal tissue and its blood vessels leads to hyaline degeneration of the connective tissue and in some instances to a complete obliteration of the periodontal membrane.

References

1. Erdheim, J. Rachitis und Epithelkörperchen. *Denkschr. d. k. Akad. d. Wissensch., Math.-naturw. Klasse, Wien,* 1914, **90,** 363–683.
2. Becks, H., and Ryder, W. B. Experimental rickets and calcification of dentin. *Arch. Path.,* 1931, **12,** 358–386.
3. McLean, F. C. The pathogenesis of rickets. *Tr. A. Am. Physicians,* 1936, **51,** 144–147.
4. Weinmann, J. P., and Schour, I. Experimental studies in calcification. I. The effect of a rachitogenic diet on the dental tissues of the white rat. *Am. J. Path.,* 1945, **21,** 821–831.
5. Sicher, H., and Weinmann, J. P. Bone growth and physiologic tooth movement. *Am. J. Orthodontics & Oral Surg.,* 1944, **30,** 109–132.
6. Hoffman, M. M., and Schour, I. Quantitative studies in the development of the rat molar. II. Alveolar bone, cementum and eruption. *Am. J. Orthodontics & Oral Surg.,* 1940, **26,** 854–874.
7. Pommer, G. Untersuchungen über Osteomalacie und Rachitis. F. C. W. Vogel, Leipzig, 1885.
8. Howland, J. The etiology and pathogenesis of rickets. *Harvey Lectures,* 1922–23, **18,** 189–216.

9. Dodds, G. S., and Cameron, H. C. Studies on experimental rickets in rats. IV. The relation of rickets to growth, with special reference to the bones. *Am. J. Path.*, 1943, **19**, 169–185.
10. Gottlieb, B. Die diffuse Atrophie des Alveolarknochens. *Ztschr. f. Stomatol.*, 1923, **21**, 195–262.
11. Orbán, B., and Weinmann, J. Die ursächlichen Bedingungen für den Abbau der Hartsubstanzen. *Virchows Arch. f. path. Anat.*, 1928, **267**, 446–455.
12. Kronfeld, R. Spielt die Qualität der Hartsubstanzen bei der Resorption eine Rolle? *Ztschr. f. Stomatol.*, 1927, **25**, 1099–1109.
13. McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. Studies on experimental rickets. VIII. The production of rickets by diets low in phosphorus and fat-soluble A. *J. Biol. Chem.*, 1921, **47**, 507–527.
14. Sicher, H. Der histologische Bau der Meerschweinchenmolaren und ihres Befestigungsapparates. I. Bau und Funktion des Fixationsapparatus der Meerschweinchenmolaren. *Ztschr. f. Stomatol.*, 1923, **21**, 580–594.
15. Steenbock, H., and Black, A. Fat soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. *J. Biol. Chem.*, 1925, **64**, 263–298.

[*Illustrations follow*]

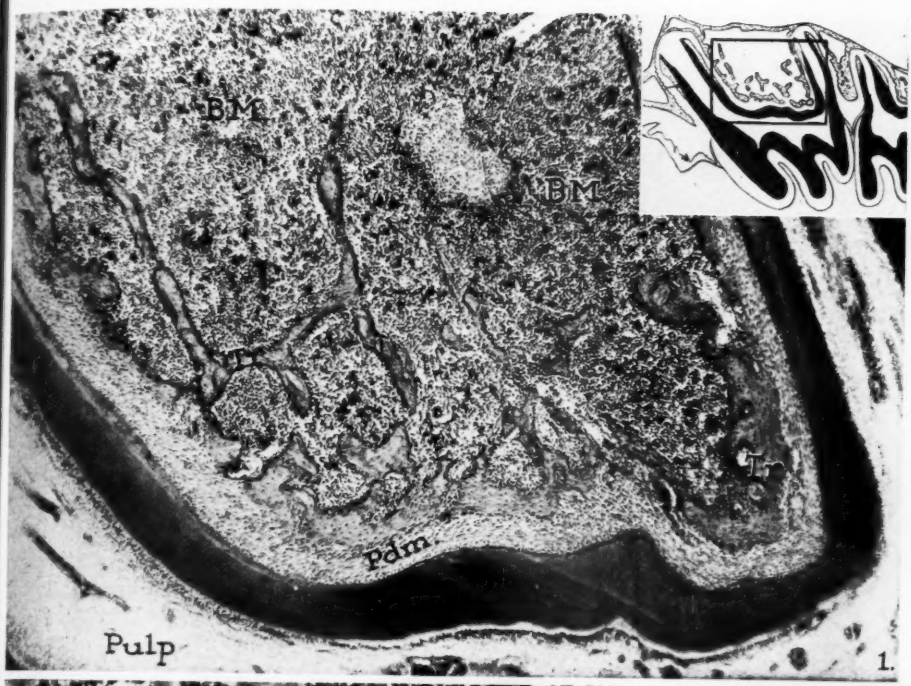
DESCRIPTION OF PLATES

PLATE 137

Photomicrographs of a mesiodistal section of the interradicular septum of a first upper molar. This area is indicated in the insert in Figure 1. × 58.

FIG. 1. Normal control rat 2901, 49 days old. Section shows thin, calcified bone trabeculae, Tr., the large marrow spaces, BM, and the even width of the periodontal membrane (Pdm.).

FIG. 2. Rat 1202, 52 days old. This animal was weaned at 21 days and then placed on the rachitogenic diet for 31 days. Section shows increased thickness of predentin (PD), cementoid (CD), and osteoid (Od.), narrowing of the marrow spaces (BM), and almost complete reduction of the periodontal membrane (Pdm.).



Weinmann and Schour Effect of Rachitogenic Diet on Alveolar Bone

PLATE 138

Photomicrographs of a midsagittal section of the lingual alveolar bone of the upper incisor. This area is indicated in the insert in Figure 5.

FIG. 3. Normal control rat 2901, 49 days old. The homogeneous calcification of the thin bone trabeculae (Tr) and large marrow spaces (BM) are to be noted. P.d.m. = periodontal membrane. × 52.

FIG. 4. Rat 1202, 52 days old, placed on the rachitogenic diet for 31 days after weaning. There is a thick layer of osteoid tissue (Od.) and the marrow spaces (BM) are reduced. × 52.

FIG. 5. Normal control rat 3601. Magnified area of periodontal membrane. Of note are the thin layer of cementum (Ct) and the division of the principal fibers into alveolar (Ar) and dental (Dl) fibers, and intermediate plexus (I.P.) Al.B. = alveolar bone. × 160.

FIG. 6. Rat 1802, 67 days old, placed on the rachitogenic diet for 46 days after weaning. Field corresponding to that of Figure 5, showing wide layer of cementoid tissue (Cd.) and the direct course of the principal fibers (P.F.) from alveolar bone (Al.B.) to cementum. × 160.

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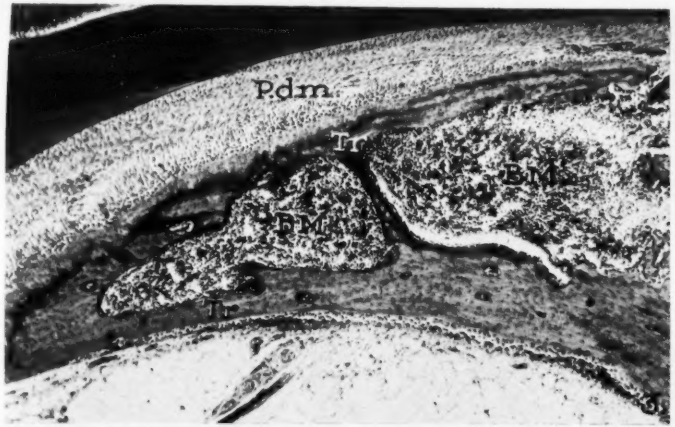

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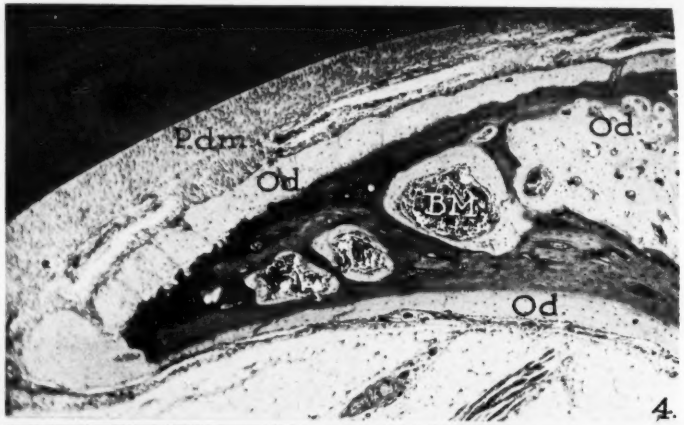

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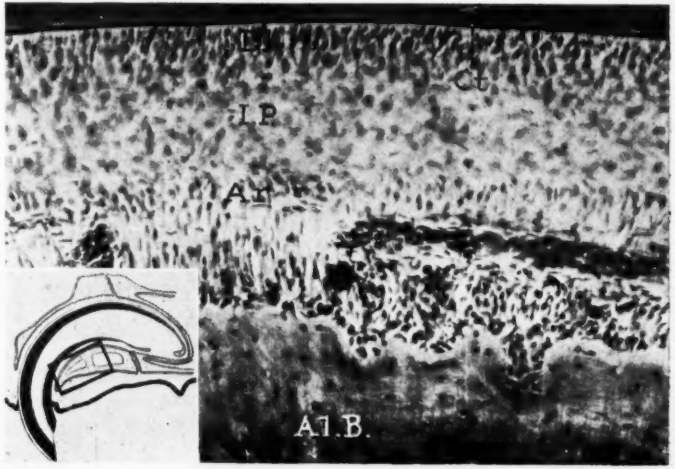

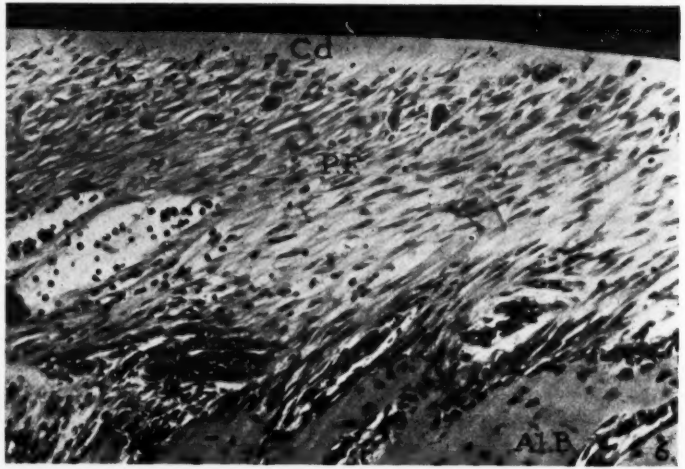

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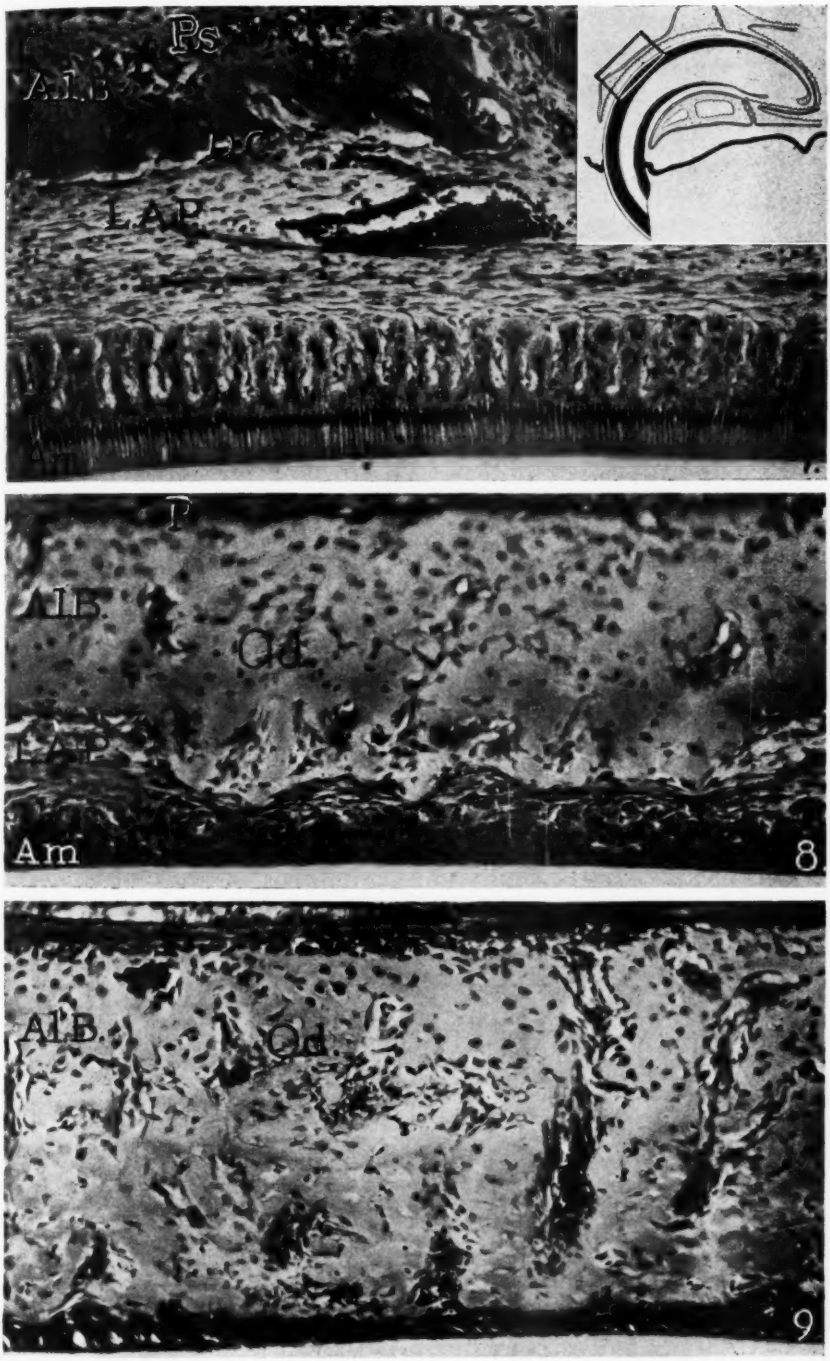
PLATE 139

Photomicrographs of decalcified midsagittal section of the labial alveolar periosteum of mid-region of upper incisor. This area is indicated in the insert in Figure 7. Am. = ameloblasts; Al.B. = alveolar bone.

FIG. 7. Normal control rat 2901, 49 days old. There is apposition of bone at the periosteal (Ps) and resorption at the dental (D.C.) surface of the alveolar bone. The width of the alveolar bone and the labial alveolar periosteum (L.A.P.) may be compared with that of Figures 8 and 9. × 172.

FIG. 8. Rat 1802, 67 days old, placed on the rachitogenic diet for 46 days after weaning. To be noted are the presence of osteoblasts on the periosteal surface (P); the absence of resorption at the dental surface of the thickened alveolar bone, which now consists of osteoid tissue (Od.); and the compression and hyaline degeneration of the labial alveolar periosteum, L.A.P. × 172.

FIG. 9. Rat 2902, 49 days old, placed on the rachitogenic diet for 28 days after weaning. Of note here are the increased thickness of the alveolar bone (Al.B.), the replacement of the labial alveolar periosteum by osteoid (Od.) and hyaline connective tissue; and the compression of the enamel organ. × 172.



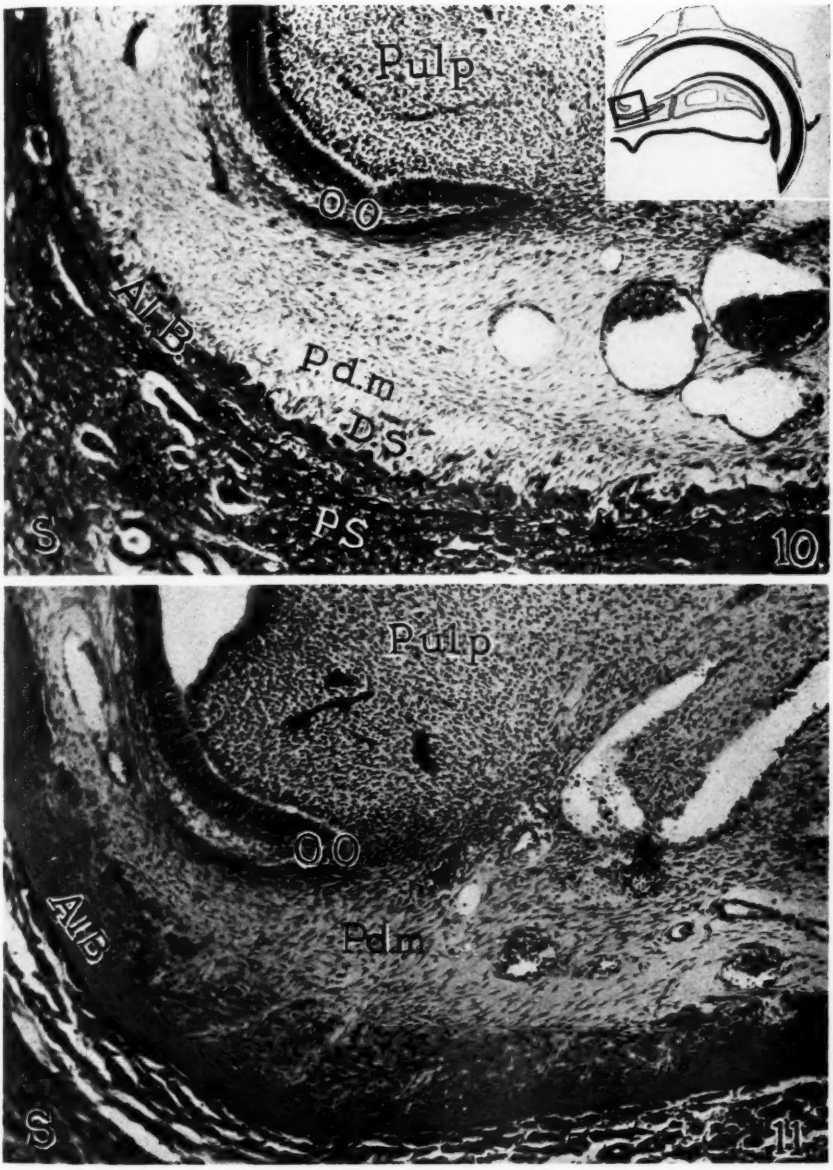
Weinmann and Schour Effect of Rachitogenic Diet on Alveolar Bone

PLATE 140

Photomicrographs of decalcified midsagittal section of the labial fundic portion of the upper incisor. This area is indicated in the insert in Figure 10. Al.B. = fundic alveolar bone; O.O. = odontogenic organ; P.d.m. = periodontal membrane; S = mucous gland. ×120.

FIG. 10. Normal control rat 102, 53 days old. Apposition of bone at the periosteal surface (P.S.) and resorption at the dental surface (D.S.) of the alveolar plate.

FIG. 11. Rat 403, 53 days old, placed on the rachitogenic diet for 32 days after weaning. Reduction of the periodontal membrane (P.d.m.) in width; lack of calcification and increase of the bony plate (Al.B) in thickness; and partial replacement of the periodontal membrane by hyaline tissue are the significant features.

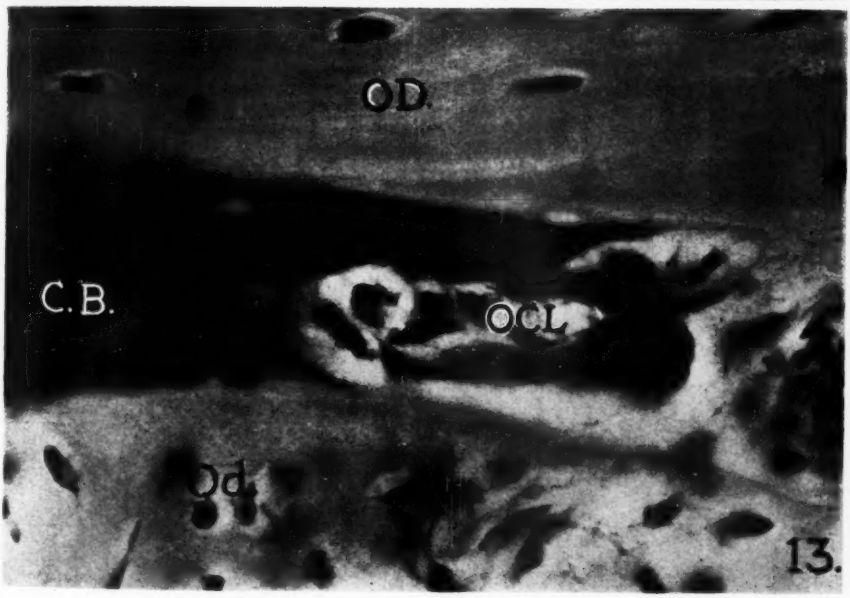


Weinmann and Schour Effect of Rachitogenic Diet on Alveolar Bone

PLATE 141

FIG. 12. Photomicrograph of midsagittal section of the labial alveolar bone of upper incisor of rat 2902, 49 days of age, which was weaned when 21 days old and fed the rachitogenic diet for 28 days. This area is indicated in the insert. Core of trabeculae consists of calcified bone (CB) and peripheral layers of osteoid tissue (Od.). × 150.

FIG. 13. Higher magnification of the area indicated in Figure 12, showing osteoclastic (OCL) resorption of the calcified core (CB) of the bone trabecula and resistance of the osteoid tissue (Od.) to resorption. × 800.



Weinmann and Schour Effect of Rachitogenic Diet on Alveolar Bone

UNIV. OF MICH.

EXPERIMENTAL STUDIES IN CALCIFICATION

III. The Effect of Parathyroid Hormone on the Alveolar Bone and Teeth of the Normal and Rachitic Rat *

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In a preceding report [1] we have shown that experimental rickets is characterized by a lack of calcification of the bone matrix. The uncalcified or osteoid tissue proved to be almost immune to osteoclastic resorption. The present study was undertaken to observe the reaction of the osteoid tissue of rachitic animals in respect to the osteosclerosis and osteoclasis of hyperparathyroidism and the response of the enamel and dentin under these conditions.

Review of the Literature

Effect of Parathyroid Hormone on the Normal Bone. Bodansky, Blair, and Jaffe [2] and Jaffe and Bodansky [3] were the first to produce experimental osteitis fibrosa in guinea-pigs and dogs by injecting large doses of parathyroid hormone. They observed extensive resorption of both cortical and spongy bone and fibrous transformation of bone marrow. In growing rats, injection of parathyroid hormone resulted in osteoporosis according to Waltner [4] and Johnson,[5] and in osteosclerosis according to Bauer, Aub, and Albright.[6] Selye,[7] Shelling, Asher, and Jackson [8] and Shelling [9] explained these apparently contradictory results on the basis of the differences in the dosage of the injected hormone. Small doses produced osteosclerosis; large doses, osteoporosis. Selye suggested that the stimulating effect of the hormone on the formation of osteoblasts may be responsible for the so-called "parathyroid hormone immunity." Burrows [10] regarded the different reactions of the bone as different stages of a continuous process. McLean and Bloom,[11] by injecting extremely large doses of parathyroid hormone, showed that osteitis fibrosa appears to be a reaction to the death or injury of the cellular elements of bone.

Effect of Parathyroid Hormone on the Normal Dentition. Schour, Tweedy, and McJunkin [12] described the effect of single and multiple injections of parathyroid hormone on the incisor of the white rat. They found hypoplasia of the enamel, osteoclasis of the alveolar bone, and fibrous replacement of bone marrow. The dentin showed a primary hypocalcified layer as the immediate effect of the first injection and a

* Received for publication, September 5, 1944.

secondary hypercalcified layer that varied with the number and dosage of the hormonal treatments. The enamel and dentin showed no resorption. Schour and Ham [13] found that the poorly calcified layer of dentin developed while the serum calcium level was rising, and that the well calcified layer developed during the period when the serum calcium level was falling.

Effect of Parathyroid Hormone on the Rachitic Bone. Shelling, Asher, and Jackson[8] found that parathyroid hormone in doses of 10 units daily failed to protect the animals against rickets. They found a moderate amount of submetaphyseal sclerosis but the newly formed bone consisted of poorly calcified trabeculae. McLean and McCoy [14] found that doses of parathyroid hormone sufficiently large to cause osteoporosis led to a dense deposit of calcium and phosphate in rachitic cartilage.

The effect of parathyroid hormone on dentition in rachitis has not been reported as far as we were able to ascertain.

Material and Method

This study is based on the histologic sections of the upper jaws of two groups of white rats.* Group I consisted of 23 control animals that were fed a normal basal diet (Table I). Group II consisted of 38 animals that were placed on the rachitogenic diet of Steenbock and Black [15] (Table II). At ages of 48 to 52 days the animals of both groups were injected with parathyroid hormone. The doses varied from 1 to 24 in number and from 50 to 780 Hanson units in quantity. The animals were killed from 1 to 132 hours after the last injection and their ages at death ranged from 48 to 73 days. An additional control group consisted of 16 normal and 17 rachitic rats of similar age.

The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin.

Findings

I. Alveolar Bone

The effect of the injection of parathyroid hormone on bone depends primarily upon the number of units in each single dose and secondarily upon the total number of units when multiple injections are used. The

* The animals used in this series of studies were kindly made available by Drs. F. C. McLean and W. Bloom.

difference in the effects of an injection of 50 Hanson units and 100 or more Hanson units is qualitative rather than quantitative.

A. The Effect of Small Doses (50 Hanson Units)

Animals Placed on Normal Basal Diet. The alveolar process of an animal injected with three daily doses of 50 Hanson units each showed the signs of increased osteoclastic and osteoblastic activity. Some of the osteoclasts had disappeared and the Howship's lacunae in some places were covered by newly formed bone. With the increase in the number of injections, a major portion of the large marrow space of the normal alveolar process (Fig. 1) was gradually replaced by a network of well calcified bone trabeculae (Fig. 2). These were densely bordered by apparently very active osteoblasts. The newly formed bone was more intensively stained than were the pre-experimental bone trabeculae. The marrow lined by the newly formed bone trabeculae was fibrous. Only small areas of red marrow were left.

Rachitic Animals. The reaction of rachitic bone to the injection of small doses (Table II) was similar to that seen in the normal animals but was less intense. A significant change could be noticed after nine daily injections of 50 Hanson units each. The osteogenic activity of the endosteum and periosteum appeared markedly stimulated. After the 18th day of the experiment the width of the osteoid border on the basal plate and along the trabeculae of the spongy bone had increased. The large bone marrow spaces of the rachitic animal (Fig. 3) appeared to be almost obliterated in the animal treated with parathyroid extract (Fig. 4).

The changes in the alveolar process were similar in the regions of the upper incisor and of the molar.

B. The Effect of Large Doses (100 or More Hanson Units)

Animals Placed on Normal Basal Diet. The number of osteoclasts increased following a single injection of 200 units of parathyroid hormone (Figs. 5 and 6). The osteoclasts were found along the trabeculae in the small as well as the large marrow spaces and along the periodontal surface of the alveolar bone. They were missing at the crest of the septa. In the areas where osteoclasts were seen, the bone was scalloped in outline (Howship's lacunae) but did not differ in staining from bone which was covered by resting and inactive periosteum or endosteum.

The number of osteoclasts and Howship's lacunae increased with an increasing total dosage of parathyroid hormone, when the single dose was 100 units or more. In the molar region, the destructive process

TABLE I

Data on 23 Rats That Were Fed a Normal Diet and Were Given Injections of Parathyroid Hormone

Rat number	Age at death	Duration of experiment	Dosage in units (Hanson)	Number of injections	Total units	Hours between last injection and death	Resorption of alv. process		Hyperosteogenesis
							Molar region	Incis. region	
3102	52 days	3 days	50	3	150		N	N	+
3103	55 days	6 days	50	6	300		N	N	+
3104	58 days	9 days	50	9	450		N	N	++
3105	61 days	12 days	50	12	600		N	N	++
3106	64 days	15 days	50	15	750		N	N	++
3107	67 days	18 days	50	18	900		N	N	++
3108	70 days	21 days	50	21	1050		N	N	++
3109	73 days	24 days	50	24	1200		N	N	++
3002	49 days	9 hrs.	200*	5	1100	5	N	N	N
3003	49 days	12 hrs.	200*	5	1100	8	N	N	N
3602	53 days	1 day	100	2	200	24	+	+	N
2302	53 days	1 day	100	2	200	24	+	+	N
103	50 days	1 day	100	2	300	0	+	N	N
2303	54 days	2 days	100	4	400	24	+	+	N
3603	54 days	2 days	100	4	400	24	+	+	N
104	51 days	3 days	100†	4	500	0	++	N	N
2304	55 days	3 days	100	6	600	24	++	±	N
3604	55 days	3 days	100	6	600	24	++	+	N
105	52 days	4 days	100†	6	700	0	+++	+	N
3605	56 days	4 days	100	8	800	24	+++	+	N
106	53 days	5 days	100†	8	900	0	++++	+	N
3606	57 days	5 days	100	10	1000		+++	++	N
3005	53 days	5 days	200*	5	1100	82	N	N	N

N = No recognizable deviation from normal.

* First injection was 300 units.

† First injection was 200 units.

Data on 38 Rats That Were Fed a Rachitogenic Diet and Given One or Multiple Injections of Parathyroid Hormone

									Histologic findings

Data on 38 Rats That Were Fed a Rachitogenic Diet and Given One or Multiple Injections of Parathyroid Hormone

Rat number	Weight at death	Sex	Age at death	Duration of experiment	Dosage units	Times injected	Total units	Hours between last injection and death	Histologic findings		
									Resorption of alveolar process		Hyper-osteogenesis
									Molar region	Incis. region	
	(*gm.*)		(*days*)								
1803	85		52	3 days	50	3	150	24	N	N	N
1804	89	M	55	6 days	50	6	300	24	N	N	N
1805	84	F	58	9 days	50	9	450	24	N	N	+
1806	111	M	61	12 days	50	12	600	24	N	N	+
1807		M	64	15 days	50	15	750	24	N	N	+
1808	73	M	67	18 days	50	18	900	24	N	N	+
1502	58	M	48	1 hr.	200	1	200	1	N	N	N
1303		M	49	1 hr.	480	1	480	1	N	N	N
1503	57	M	48	2 hrs.	200	1	200	2	N	N	N
1304		M	49	2 hrs.	780	1	780	2	N	N	N
1504	62	F	48	3 hrs.	200	1	200	3	N	N	N
1305		F	49	4 hrs.	600	1	600	4	N	N	N
1506		M	48	8 hrs.	200	1	200	8	N	N	N
1306		F	49	8 hrs.	740	1	740	8	N	N	N
3505		M	50	1 day	100	1	100	24	N	N	N
1203	65	F	49	1 day	160	1	160	24	N	N	N
304	66	F	50	1 day	200	1	200		N	N	N
1604		M	50	1 day	200	1	200	24	N	N	N
1205	69	M	50	1½ days	180	1	180	36	+	N	N
3506		F	51	2 days	100	2	200	24	+	N	N
1605			51	2 days	200	2	400	48	+	N	N
25			50	2 days	100	5	500	6	++	N	N
27			50	2 days	100	5	500	6	+	N	N
1206	72	F	51	2½ days	200	1	200	60	+	N	N
3507		F	52	3 days	100	3	300	24	+	N	N
703	74	M	52	3 days	100	4	400	0	N	N	N
306	47	F	52	3 days	200	3	600		++	±	N
1606	95	M	52	3 days	200	3	600	24	++	N	N
1207	76	M	52	4 days	180	1	180	84	N	N	N
3508			53	4 days	100	4	400	24	+	N	N
307	55	F	53	4 days	200	4	800		++	N	N
1607		M	54	4 days	200	4	800	4	++	+	N
1208	72	M	53	5 days	190	1	190	108	N	N	N
1209	82	F	54	6 days	200	1	200	132	N	N	N
704	74		55	6 days	100	7	700	24	++	N	N
305	61	F	57	8 days	200	2	400		+	N	N
705	90		59	9 days	100	9	900	24	++	++	N
706	88		62	11 days	100	11	1100	24	++	+	N

N = No recognizable deviation from normal.

affected, first, the basal portion (Fig. 6) of the alveolar process and, later, the alveolar crest (Fig. 7). Daily injections of 200 units led to a complete disappearance of the alveolar bone after 5 days (Fig. 8). The alveolar bone was then replaced by connective tissue containing numerous fibroblasts, osteoclasts, and capillaries. The osteoclasts and fibroblasts were grouped in strands. A large part of the red bone marrow and of the periodontal membrane appeared unchanged even in the most advanced stages of hyperparathyroidism. The principal fibers of the periodontal membrane could be traced into the fibrous tissue which had replaced the bone trabeculae (Fig. 8).

The increase of osteoclasts was not noticed until 24 hours following a single injection of large dosage. Even the injection of massive doses of 1100 units did not cause an increase of osteoclasts within 12 hours. The reaction disappeared on the fourth day following a single injection.

Rachitic Animals. The reaction of rachitic alveolar bone to the injection of large doses of parathyroid hormone differed from that of normal bone only in degree (Table II). Following the injection of 200 or more Hanson units of parathyroid hormone, large numbers of osteoclasts were present in the alveolar process of the molar region. Twenty-four hours after the injection, fibrous tissue replaced part of the bone trabeculae. Most of the osteoclasts were found adjacent to the calcified part of the trabeculae (Fig. 10). A comparison of the alveolar bone of the rachitic and of the normal rat after the injection of the same doses of parathyroid hormone revealed that osteoclasis was far more advanced in the calcified portion than in osteoid tissue (compare Figs. 8 and 10). The osteoid portion of the trabeculae resisted resorption much longer than did the calcified portion.

The reaction of the alveolar bone was less intense in the upper incisor area than in the molar region. This was true for the normal (Figs. 11 and 12) as well as for the rachitic animal. In animals placed on a basal diet and given injections of parathyroid hormone, the bony plates of the alveolar bone of the incisors were reduced in thickness. In no site was there as complete a replacement by fibrous tissue as in the alveolar process of the molars, not even in those areas where internal resorption is normally found and where the bony plate is exceedingly thin. In rachitic animals the alveolar bone of this area showed only slight changes which could be attributed to the injection of parathyroid hormone.

II. DENTAL TISSUES

Upper Molars. The enamel and dentin of the upper molars showed no resorption and the incidence of root resorption was not significantly different from that found in the control animals. The recently formed dentin showed a hypercalcified stripe.

Upper Incisor. Injection of 50 Hanson units of parathyroid hormone, even repeated as many as 24 times, did not cause significant changes in the tissues of the incisors. Multiple injections, each of 100 or more Hanson units, in the control rats placed on a basal diet resulted in a stratification of the dentin. The dentin calcifying on the first day following the injection remained hypocalcified and appeared as a pink stripe which had the same width as the predentin. The later dentin was hypercalcified and stained intensively with hematoxylin. The striation was more pronounced in the middle and incisal thirds of the labial dentin. These observations are in complete agreement with the findings of Schour and his collaborators.[12, 13]

The changes in the dentin of rachitic rats following injections of parathyroid hormone were of questionable significance. In the incisal third the layer of dentin next to the predentin stained deeply with hematoxylin, indicating some hypercalcification. The uncalcified basal end (basal predentin) of the incisor seemed to be shorter. On the other hand, the width of the predentin and the frequency of the intermediate dentin were the same as in the control rachitic rats.

The injections of parathyroid hormone had no significant effect on the enamel organ or the enamel.

Discussion

The Paradoxic Effects of Small and Large Doses of Parathyroid Hormone

Our observations on the alveolar bone are in agreement with the findings in long bones reported by Shelling, Asher, and Jackson [8] and by Selye[7] who found that massive doses produce osteitis fibrosa, but small doses cause osteoblastic activity and an increase in the number of bone trabeculae. Shelling and his collaborators refrained from explaining this paradoxic reaction. Burrows [10] distinguished three phases in the effect of parathyroid hormone. The first phase is characterized by the resorption of bone. In the second phase osteoblasts reappear and excessive bone deposition occurs. In the third stage there is a tendency of the bone to return to a normal state. Burrows, in agreement with Selye's explanation, regards the sequence of changes as "due to the development of an antibody-like substance in the blood which acts as a check on decalcification and in its stead introduces hypercalcification."

As far as our material permitted, we were able to differentiate two stages, but only after injections of small doses of parathyroid hormone. The osteoblastic activity followed a short period of increased osteoclasis. It led to osteosclerosis. A return to normalcy could not be observed in our animals. We agree with the explanation offered by Selye [7]

and Burrows [10] that injections of parathyroid hormone of bovine origin might cause the formation of an antibody in the rat. After a short interval of osteoclasis these antibodies could be produced in excess and would neutralize not only the injected hormone but also some of the parathyroid hormone produced by the glands of the animal. However, with the injection of massive doses the production of antibodies is not sufficient to overcome the surplus of hormone. Small doses would in this way lead in the end to hypoparathyroidism and large doses would produce the symptoms of hyperparathyroidism.

Experimental proof of this hypothesis is not yet available, but the clinical observations tend to support it. Aub, Albright, Bauer, and Rossmeisl [16] found that parathyroid extract gradually loses its therapeutic effect in tetany after long-continued administration. They assumed that the patient acquired active immunity to the injected hormone. Furthermore, Kendall, Walsh, and Howard [17] observed that idiopathic hypoparathyroidism causes overproduction in bone as shown by its increased density in roentgenograms.

Osteoclasis in hyperparathyroidism should be regarded as a pathologic exaggeration of the physiologic process of bone resorption which is an integral factor in bone development and bone growth. Resorptive processes are entirely lacking in the development and growth of the tooth, with the exception of the shedding of deciduous teeth. This might explain the freedom of enamel, dentin, and cementum from resorption in hyperparathyroidism.

Reaction of Rachitic Bone to Parathyroid Hormone

The reactions of rachitic and of normal bone to injection of parathyroid hormone were essentially the same. In both instances new bone formation was stimulated by small doses and resorption of bone was caused by large doses. A comparison of the two groups of experiments, however, showed a definite difference in the reaction following injection of large doses. In all instances the resorption of the rachitic bone was less advanced than was the resorption of bone of the normally fed rats under otherwise similar conditions. The explanation for this striking difference is found in the fact that bone which is uncalcified (osteoid tissue) is more resistant to resorption than calcified bone. That the degree of calcification might play a rôle in the susceptibility to resorption was suggested by Gottlieb [18] and substantiated by Orbán and Weinmann [19] and Kronfeld.[20] Strong support of the theory is seen in the findings reported in the preceding communication [1] by which it could be demonstrated that osteoid tissue is relatively immune to resorption.

Differences in Reaction of the Alveolar Bone of Molar Region and of Incisor Region

Differences in reaction of various areas of bone to the parathyroid hormone were observed by Jaffe [21] who stated that "the sites of most active bone formation are the sites most susceptible to resorption." It is likely that the greater resorptive activity in the alveolar process of the molar area as compared with that in the region of the incisor is a result of the greater growth activity of the former.

Summary and Conclusions

The effect of single and multiple injections of parathyroid hormone on the upper jaws of the white rat was studied histologically in 23 animals fed a normal diet and in 38 animals placed on a rachitogenic diet after weaning. Sixteen normal and 17 rachitic rats from 42 to 77 days of age were used as controls. The histologic findings were:

1. Injections of 50 Hanson units of parathyroid hormone stimulated osteoblastic activity and new formation of alveolar bone.

2. Injections of 100 or more Hanson units of parathyroid hormone resulted in progressive osteoclasis of the bone and replacement of the bone by fibrous tissue.

3. The reaction of the bone to parathyroid hormone was essentially the same in normal and rachitic rats, except for the fact that the osteoid tissue in the latter was more resistant to resorption.

4. Following injections of large doses of parathyroid hormone, the alveolar bone of the molar areas showed more severe changes than that of the incisor region.

5. Injections of parathyroid hormone do not cause resorption of the hard dental structures (enamel, dentin, and cementum).

References

1. Weinmann, J. P., and Schour, I. Experimental studies in calcification. II. The effect of a rachitogenic diet on the alveolar bone of the white rat. *Am. J. Path.*, 1945, **21**, 833–855.
2. Bodansky, A., Blair, J. E., and Jaffe, H. L. Experimental hyperparathyroidism in guinea-pigs leading to ostitis fibrosa. *J. Biol. Chem.*, 1930, **88**, 629–647.
3. Jaffe, H. L., and Bodansky, A. Experimental fibrous osteodystrophy (ostitis fibrosa) in hyperparathyroid dogs. *J. Exper. Med.*, 1930, **52**, 669–694.
4. Waltner, K. Über die Funktion der Nebenschilddrüse. *Monatschr. f. Kinderh.*, 1928, **40**, 317–329.
5. Johnson, J. L. Experimental chronic hyperparathyroidism. II. Osteitis fibrosa produced in rats. *Am. J. M. Sc.*, 1932, **183**, 761–769.
6. Bauer, W., Aub, J. C., and Albright, F. Studies of calcium and phosphorus metabolism. V. A study of the bone trabeculae as a readily available reserve supply of calcium. *J. Exper. Med.*, 1929, **49**, 145–161.
7. Selye, H. On stimulation of new bone formation with parathyroid extract and irradiated ergosterol. *Endocrinology*, 1932, **16**, 547–558.

8. Shelling, D. H., Asher, D. E., and Jackson, D. A. Calcium and phosphorus studies. VII. The effects of variations in dosage of parathormone and of calcium and phosphorus in the diet on the concentrations of calcium and inorganic phosphorus in the serum and on the histology and chemical composition of the bones of rats. *Bull. Johns Hopkins Hosp.*, 1933, **53**, 348–389.
9. Shelling, D. H. The Parathyroids in Health and in Disease. C. V. Mosby Co., St. Louis, 1935.
10. Burrows, R. B. Variations produced in bones of growing rats by parathyroid extracts. *Am. J. Anat.*, 1937–38, **62**, 237–290.
11. McLean, F. C., and Bloom, W. Mode of action of parathyroid extract on bone. *Science*, 1937, **85**, 24.
12. Schour, I., Tweedy, W. R., and McJunkin, F. A. The effect of single and multiple doses of the parathyroid hormone on the calcification of the dentin of the rat incisor. *Am. J. Path.*, 1934, **10**, 321–342.
13. Schour, I., and Ham, A. W. Action of vitamin D and of the parathyroid hormone on the calcium metabolism. *Arch. Path.*, 1934, **17**, 22–39.
14. McLean, F. C., and McCoy, R. H. Calcification in rachitic cartilage induced by administration of phosphate, and by parathyroid extract. *J. Biol. Chem.*, 1936, **114**, lxv–lxvi.
15. Steenbock, H., and Black, A. Fat soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their nonsaponifiable constituents by exposure to light. *J. Biol. Chem.*, 1925, **64**, 263–298.
16. Aub, J. C., Albright, F., Bauer, W., and Rossmeisl, E. Studies of calcium and phosphorus metabolism. VI. In hypoparathyroidism and chronic steatorrhea with tetany with special consideration of the therapeutic effect of thyroid. *J. Clin. Investigation*, 1932, **11**, 211–234.
17. Kendall, E., Jr., Walsh, F. B., and Howard, J. E. Idiopathic hypoparathyroidism. *Ann. Int. Med.*, 1941, **14**, 1256–1270.
18. Gottlieb, B. Zur Ätiologie und Therapie des Alveolarpyorrhoe. *Ztschr. f. Stomatol.*, 1920, **18**, 59–82.
19. Orbán, B., and Weinmann, J. Die ursächlichen Bedingungen für den Abbau der Hartsubstanzen. *Arch. f. path. Anat.*, 1928, **267**, 446–455.
20. Kronfeld, R. Spielt die Qualität der Hartsubstanzen bei der Resorption eine Rolle? *Ztschr. f. Stomatol.*, 1927, **25**, 1099–1109.
21. Jaffe, H. L. Hyperparathyroidism (Recklinghausen's disease of bone). *Arch. Path.*, 1933, **16**, 63–112; 236–258.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 142

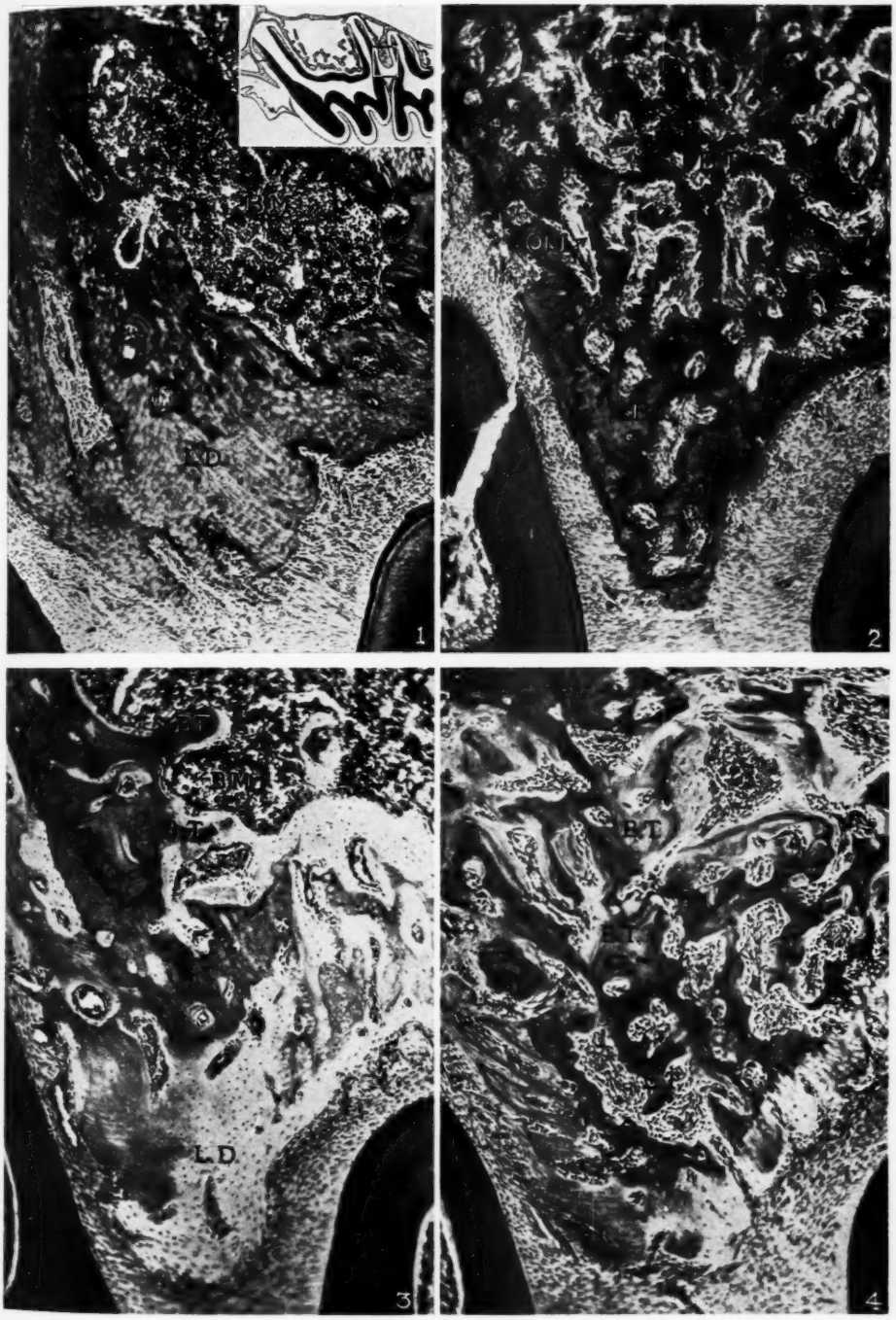
Photomicrographs of decalcified mesiodistal sections of the interdental septum between the first and second upper molars showing the effect of small doses of parathyroid hormone on the normal and rachitic alveolar bone. Area is indicated in the insert in Figure 1. B.M. = bone marrow; B.T. = bone trabeculae. × 70.

FIG. 1. Rat 1801, 67 days old. Normal control. The lamina dura (L.D.) is thick and well calcified and the bone marrow spaces (B.M.) are large.

FIG. 2. Rat 3109, 73 days old. Normal diet. Twenty-four daily injections of parathyroid hormone, each 50 Hanson units, were administered. A network of calcified bone trabeculae (B.T.) replaces the pre-experimental bone and fills a large part of the bone marrow spaces. The bone trabeculae are bordered by osteoblasts (Obl.). At a few places osteoclasts can be seen.

FIG. 3. Rat 1802, 67 days old. Rachitogenic diet. The width of the lamina dura (L.D.) and of the bone trabeculae (B.T.) is increased. The core of the bone trabeculae is calcified and covered by thick layers of osteoid tissue. The bone marrow spaces (B.M.) are narrow.

FIG. 4. Rat 1808, 67 days. Rachitogenic diet for 46 days after weaning. Eighteen daily injections of parathyroid hormone, each of 50 Hanson units, were administered. The thick lamina dura is replaced by a network of bone trabeculae which are covered by a layer of osteoid tissue and by osteoblasts, indicating progressing bone apposition. The large bone marrow spaces are partly filled by newly formed bone trabeculae (B.T.).



Weinmann and Schour Effect of Parathyroid Hormone

Plate 143

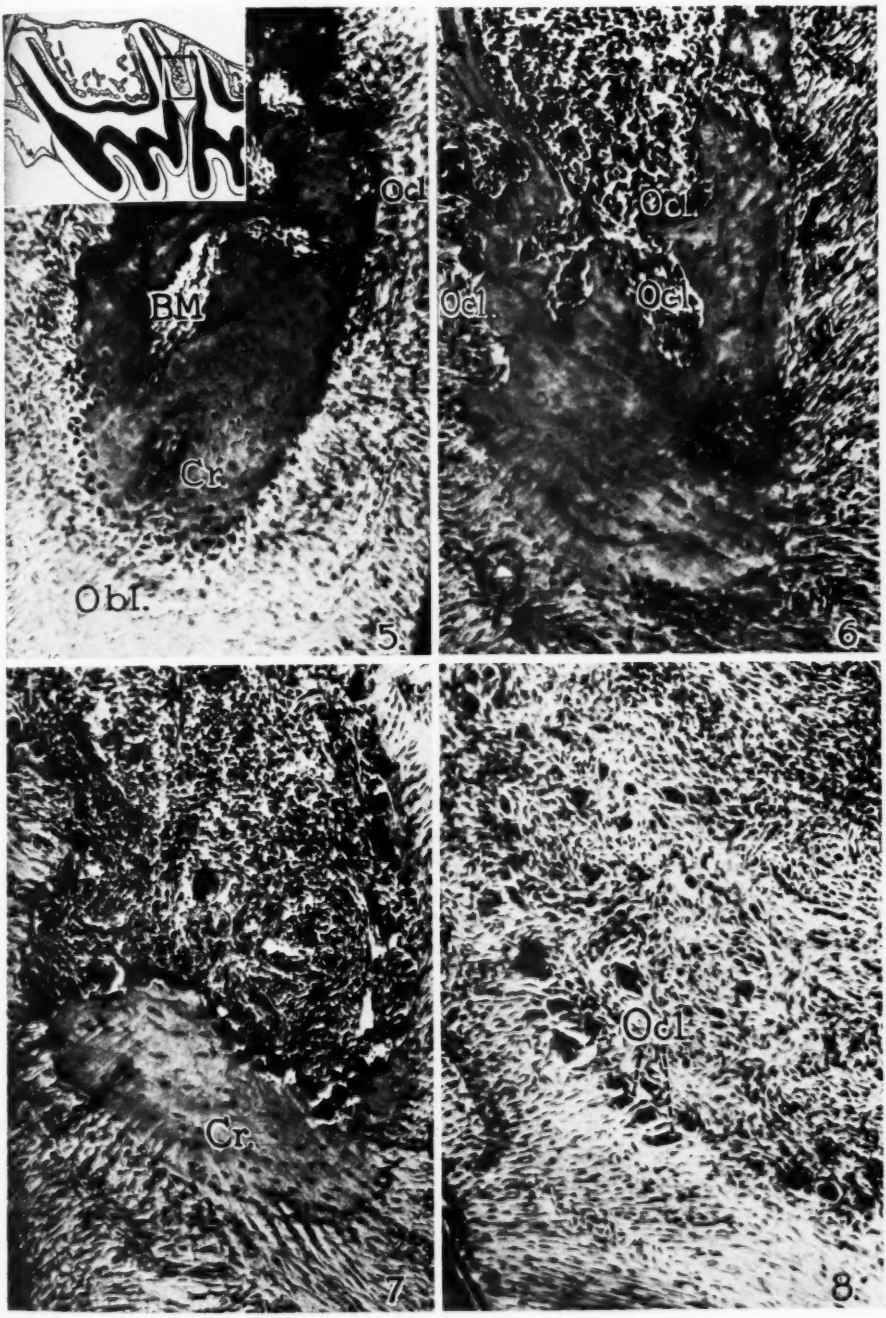
Photomicrographs of decalcified mesiodistal sections of the interdental alveolar septum between first and second upper molars showing the effect of large doses of parathyroid hormone on the alveolar bone of rats kept on a normal basal diet. Area is indicated in the insert in Figure 5. × 135.

Fig. 5. Rat 2901, 49 days old. Normal control. The bone trabeculae are well calcified, the largest forming the crest (Cr.) covered by osteoblasts (Obl.); in the lateral parts few osteoclasts (Ocl.) are seen. Bone marrow spaces (B.M.) are small.

Fig. 6. Rat 103, 50 days old. Two injections of a total of 200 Hanson units of parathyroid hormone were administered. There is an increased number of osteoclasts (Ocl.) at the periodontal and endosteal surfaces.

Fig. 7. Rat 104, 51 days old. Four injections of a total of 500 Hanson units of parathyroid hormone were administered within 3 days. Progressive osteoclasis may be noted. The alveolar bone is resorbed except for a small cap of bone at the crest (Cr.).

Fig. 8. Rat 106, 53 days old. Eight injections of a total of 900 Hanson units of parathyroid hormone were administered within 5 days. The alveolar bone is resorbed by osteoclasts (Ocl.) and replaced by fibrous tissue.



Weinmann and Schour Effect of Parathyroid Hormone

PLATE 144

Photomicrographs of decalcified mesiodistal sections of the interdental alveolar septum between first and second upper molars showing the effect of larger doses of parathyroid hormone on the alveolar bone of rats kept on rachitogenic diet. Area is indicated in the insert in Figure 9. × 140.

FIG. 9. Rat 1202, 54 days old. Rachitic control. The alveolar ridge is formed by osteoid tissue (Od.); the basal part is calcified (C.B.).

FIG. 10. Rat 307, 53 days old. Four injections, of parathyroid hormone, totalling 800 Hanson units, were administered. Resorption is active primarily in the calcified bone, which is replaced by fibrous tissue (F.T.) rich in giant cells, while the larger part of the osteoid tissue (Od.) is left intact.

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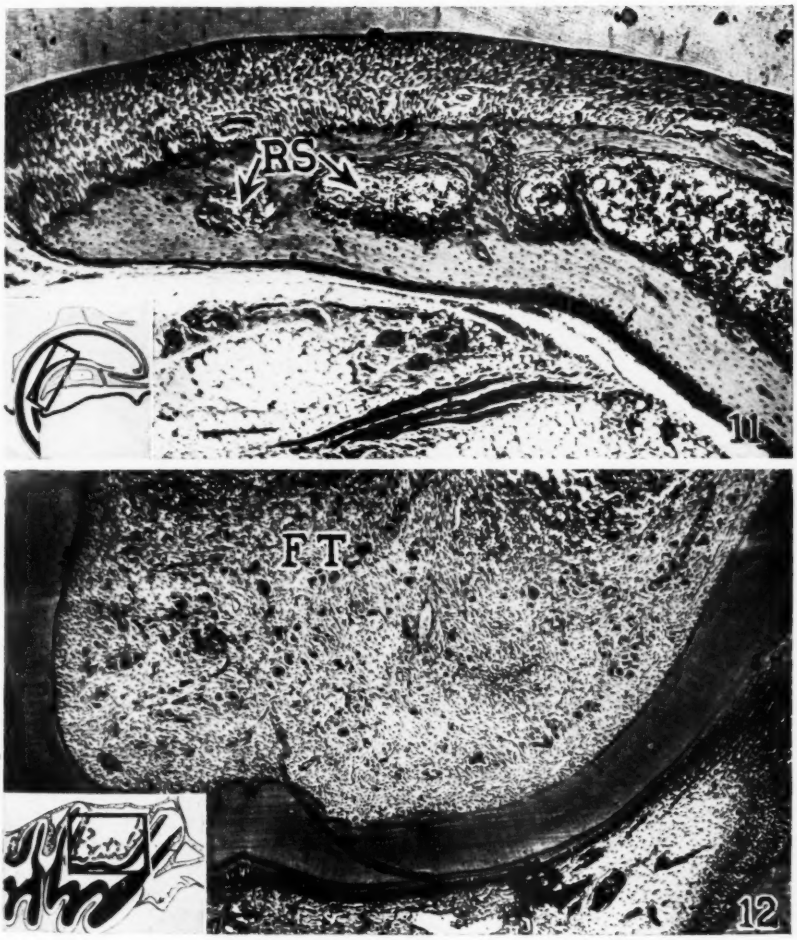
Effect of Parathyroid Hormone

PLATE 145

Photomicrographs of decalcified sections showing the different reactions of the lingual alveolar bone of the upper incisor (*Fig. 11*) and of the interradicular septum at the bifurcation of the first upper molar (*Fig. 12*) of rat 106, 53 days old, kept on the basal diet and administered a total dosage of 900 Hanson units of parathyroid hormone. While the alveolar bone of the molar is entirely resorbed and replaced by fibrous tissue (F.T.), the alveolar bone of the incisor shows only slight resorption (R.S.). Areas are indicated in the inserts. × 90.


RS
11
F T
12

A STUDY OF THE CIRCULATION OF THE SPLEEN IN SICKLEMIA AND SICKLE CELL ANEMIA *

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Numerous reports [1-3] are available describing the histologic changes in the spleens of Negroes whose erythrocytes showed sickled forms. No reports are found attempting to correlate these changes with the bizarre shapes of the erythrocytes. Rich [1] stated that the changes in the spleen are congenital in origin. In the study of a large number of cases of sicklemia and sickle cell anemia the failure to find evidence of congenital vascular abnormalities in the spleens, and the possibility that the splenic picture described as being typical of the sickling phenomenon may be the result of the sickled cells, led to the studies reported here.

NORMAL CIRCULATION OF THE SPLEEN

The circulation of the normal spleen has been a controversial subject and is dealt with in detail by Klemperer [4] who gives the current concept as follows:

"Because of the net-like structure of the arterial terminations, as well as of the venous sinuses, the circulation is evidently open. However, the width of the stomata is variable, and is influenced by various factors. For instance, contraction of the spleen will cause a diminution of the size of the pores, whereas increased tension within the vascular channels provoked by elevation of the arterial or venous pressure will result in dilatation of the stomata within the vascular wall. . . . It is evident that if the pores in the vascular channels are wide open, the blood will escape freely into the surrounding pulp spaces, whereas if they are narrow, the blood will remain within a pathway separated from the surrounding spongy spaces of the pulp by a perforated but very narrow meshed wall."

MacNeal, Otani, and Patterson,[5] in a series of injection preparations of spleens, classified the capillaries of the spleen into three groups: (1) follicular capillaries, (2) capillaries of marginal zone, and (3) capillaries of the pulp cords. They felt that the follicular capillaries arose from the central arteries, passed through the substance of the follicle and terminated just beyond the marginal zone by numerous small openings into the red pulp, always at considerable distances from the nearest venous sinuses. The follicular capillaries were frequently united by short branches. In some instances the endings were distended, presenting an ampulla, with restriction of erythrocytes and allowing the escape of plasma through the endothelial walls. In other instances there were large openings between the endothelial cells permitting the escape of erythrocytes into the pulp cords and, from them, into the venous sinuses through the stomata of Mollier.[6] The capillaries of the marginal zone arose from branches of the follicular capillaries passing into the (red) pulp where they branched, and the small branches terminated in

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the marginal zone of the follicle in the pulp spaces as the Schweigger-Seidel capillaries. The tips of these capillaries were usually distended to thin-walled ampullae with narrow clefts leading into the pulp spaces of the marginal zone. The capillaries of the pulp cords were branches of the capillaries of the marginal zone and terminated in the pulp spaces, with or without ampullae, and in some instances emptied directly into the venous sinuses. These authors thought that the circulation of blood in the spleen was open, with the pulp spaces constituting the connections between the arterial and venous channels.

Robinson [7] likewise came to the conclusion that the circulation of the spleen was open, and that the pulp spaces were the only communications between the arteries and veins. In regard to the arterial terminations he stated:

"... . Golz, a co-worker of Thoma, using a granular injection mass, showed that the arterioles passed through the ellipsoids and ended in ampullatous dilatations of the vessels. These findings were confirmed by Mall who designated the dilatation of the capillary as 'the ampulla of Thoma.' "

Robinson concluded that the ampullae of Thoma were the funnel-shaped capillary endings in the red pulp cords, and that those seeming to be dilated into ampullae were merely exaggerated pulp spaces.

Material

The material studied can be divided into two parts. The first part is composed of 150 autopsies in which sickled erythrocytes were found in routine sickling preparations as reported elsewhere.[8] Sections of spleen stained by hematoxylin and eosin following formol-alcohol fixation were available in all these cases, and also included are 24 cases of sickle cell anemia. The second part consists of 10 cases of sicklemia and 2 cases of sickle cell anemia diagnosed before death; the spleens of these cases were perfused by the method described by MacNeal, Otani, and Patterson.[5] Paraffin sections were stained with hematoxylin and eosin, van Gieson's mixture, and by Gomori's [9] silver impregnation method for reticulum. Digestion preparations were also made in some instances according to the method of Kyes.[10]

The 2 patients with sickle cell anemia had enlarged spleens but there was no calcium or extensive iron deposit present and they were therefore considered to be relatively early examples of sickle cell anemia, suitable for study.

Results

Routine Autopsy Sections Stained with Hematoxylin and Eosin. Sections of spleen from 150 cases of sicklemia and 24 cases of sickle cell anemia showed no evidence of congenital or other abnormalities of the arteries or arterial capillaries. "Pooling" in the marginal zone was marked in most cases; in 4 cases of sicklemia no "pooling" was present and the erythrocytes were not sickled, although sickled forms were

found in other organs from these cases. The venous sinuses were generally collapsed and the lining endothelial cells were frequently prominent in their opposing positions. The sickled erythrocytes were compressed and packed, usually in a circular, lamellated fashion with obvious overlapping, giving the impression of considerable rigidity, and they were located in the dilated pulp spaces (Fig. 1).

Arterially Perfused Preparations. Sections prepared from arterially perfused preparations showed the erythrocytes to be washed out of the arteries, arterial capillaries, and the venous sinuses. Erythrocytes were present in the pulp cords, especially in the marginal zones and these obviously represent the "pools" (Fig. 2). In this material it was easy to trace the follicular capillaries, marginal capillaries, and pulp capillaries to their terminations in the pulp cords. There were slight ampullar formations in most instances (Fig. 3), but when compared with normal controls and with the illustrations of others (MacNeal, Otani, and Patterson: [5] Plate 42, Fig. 2; Plate 43, Fig. 4) there was no evidence of abnormal dilatation, congenital defects, or of direct emptying into the venous sinuses. It was impossible, therefore, to explain the "pooling" in the marginal zones by changes in these arterial capillaries.

Preparations Following Venous Perfusion. In sections after venous perfusion, filling of the small venous sinuses with erythrocytes and emptying of the arterial channels were found to have resulted. The pulp cords contained erythrocytes and the "pools" were accounted for by these distended pulp cords. No evidence of reverse flow of erythrocytes from the pulp cords into the arterial capillaries was present. The impression obtained from these slides was that the perfused fluid carried the erythrocytes from the larger veins back into the small venous sinuses; then the fluid escaped from the venous sinuses into the pulp spaces through the stomata of Mollier,[6] and then into the arterial capillary endings in the pulp cords, washing out the erythrocytes originally in these capillaries. There were no demonstrable abnormalities in the arterial capillary terminations or ampullae and, in spite of the reversal of the circulation, no "pools" were produced in the venous sinuses (Fig. 4).

Other Preparations. In 2 cases of sicklemia, the spleens were perfused with distilled water through the arteries until the water became clear, then formol-alcohol was substituted and the spleens were fixed in a distended state. Sections revealed almost total hemolysis of the erythrocytes and the arterial and venous channels were open. No vascular abnormalities were found. The pulp cords were slightly distended but there were no "pools" present (Fig. 5). It was impossible to distinguish the sections of sicklemia cases from sections of normal spleens similarly treated. In spite of the drastic treatment, the pulp cords and other structures were remarkably well preserved.

Sections prepared in the preceding manner and also sections digested in pancreatin [10] were stained for reticulum. No abnormal content or arrangement of the reticulum was seen in the perfused sections and the capillary terminations showed no congenital abnormalities (Fig. 6). In the digested sections, which were not injected, some widening of the pulp cords was present but the results were not satisfactory for interpretation.

Discussion

Rich,[1] in describing the histologic picture of the spleen in sicklemia and sickle cell anemia, said:

"The peculiar splenic lesion characteristic of this condition, and present in latent as well as in active cases, consists in a congenital malformation of the sinuses of the spleen, which permits the free escape of blood into the pulp. The entire pulp may be so involved, but that alone is not specific, as it occurs also in congenital hemolytic jaundice. The anomaly characteristic of sickle cell anemia is the pronounced malformation of the sinuses immediately about the malpighian bodies, leading to the formation of pools of blood partially or completely surrounding the malpighian bodies. This is referred, for reasons discussed, to imperfections in the ampullae of Thoma. There is frequently, besides, an abnormal development of capillaries throughout the malpighian body. The vascular abnormalities in and immediately about the malpighian bodies may or may not be associated with the free escape of blood into the general splenic pulp. The reticulum of the spleen is not primarily abnormal."

Klemperer [4] stated:

". . . . Ono (1930) has not found closed pathways at any period of the embryonal development of the human spleen. In view of this most recent observation, it is questionable whether histogenetic anomalies can be held to account for the circulatory variations. It is important to emphasize this scepticism because of recent attempts to explain the pathology of sickle-cell anemia with the hypothesis that an anomaly of the arterial terminations in the spleen is responsible (Rich, 1928)."

Diggs [2] described the enlarged spleens of sickle cell anemia as showing the splenic cords stuffed with sickled erythrocytes and the sinusoids compressed and empty. He also stated:

"In some cases, but not in all, the capillaries of the malpighian bodies are dilated, so that they appear as multiple small varices or as one or more great pools of blood lying within or at the edge of the lymphoid aggregates."

From the results obtained in this study it is thought that there are no congenital abnormalities of the arterial capillary endings in the red pulp cords (ampullae of Thoma), of the venous sinusoids, or of the pulp cords *per se*.

The histologic picture resembles that of active chronic hyperemia, with the pulp cords, especially in the marginal zones, packed with lamellated sickled erythrocytes that do not, because of their shapes, escape readily through the stomata of Mollier.[6] The discharge of concentrated erythrocytes into the pulp cords, from the follicular and marginal capillaries, is accompanied by appreciable arterial capillary pressure which accentuates the packing of sickled erythrocytes and dilatation of the pulp cords in these cases. It is possible that by returning to their normal shape, the previously sickled erythrocytes packed

in the pulp cords would escape through the stomata of Mollier. This is the probable explanation of why more marked changes are not found in the spleens of most cases of sicklemia.

In sickle cell anemia there are always some sickled erythrocytes present *in vivo*.[11] The degree of sickling is variable, and according to some [12] is correlated with the symptoms present. When sickled erythrocytes are always present, as in sickle cell anemia, we could expect to find constantly the "pooling" and intense packing of these sickled forms. This would result, in time, in thrombosis, hemorrhage, and scarring with calcium and iron deposits, and lead eventually to the picture of the small fibrotic spleens found in older, active cases of sickle cell anemia.

Conclusions

1. Histologic examination of various preparations of spleen, obtained at autopsies from people who had sicklemia or sickle cell anemia, failed to show any congenital abnormalities of the arterial capillaries, venous sinusoids, or red pulp cords.

2. The "pooling" in the spleen, described as typical of the sickling phenomenon, occurs in the red pulp cords of the marginal zones.

3. It is thought that the "pooling" is, for reasons discussed, the result of circulating sickled erythrocytes.

REFERENCES

1. Rich, A. R. The splenic lesion in sickle cell anemia. *Bull. Johns Hopkins Hosp.*, 1928, **43**, 398–399.
2. Diggs, L. W. Siderofibrosis of the spleen in sickle cell anemia. *J. A. M. A.*, 1935, **104**, 538–541.
3. Steinberg, B. Sickle cell anemia. *Arch. Path.*, 1930, **9**, 876–897.
4. Klemperer, P. The Spleen. In: Downey, H. Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, **3**, pp. 1632, 1634.
5. MacNeal, W. J., Otani, S., and Patterson, M. B. The finer vascular channels of the spleen. *Am. J. Path.*, 1927, **3**, 111–122.
6. Mollier, S. Über den Bau der capillaren Milzvenen (Milzsinus). Eine kritische Studie und eigene Beobachtungen. *Arch. f. mikr. Anat.*, 1910–11, **76**, 608–657.
7. Robinson, W. L. The vascular mechanism of the spleen. *Am. J. Path.*, 1926, **2**, 341–355.
8. Tomlinson, W. J. The incidence of sicklemia and sickle cell anemia in 3,000 Canal Zone examinations upon natives of Central America. *Am. J. M. Sc.*, 1945, **209**, 181–186.
9. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, pp. 164–165.
10. Kyes, P. The intralobular framework of the human spleen. *Am. J. Anat.*, 1901–02, **1**, 37–43.
11. Klinefelter, H. F. The heart in sickle cell anemia. *Am. J. M. Sc.*, 1942, **203**, 34–51.
12. Hahn, E. V. Sickle-cell (drepanocytic) anemia, with report of a second case successfully treated by splenectomy and further observations on the mechanism of sickle-cell formation. *Am. J. M. Sc.*, 1928, **175**, 206–217.

DESCRIPTION OF PLATES

KEY TO FIGURES

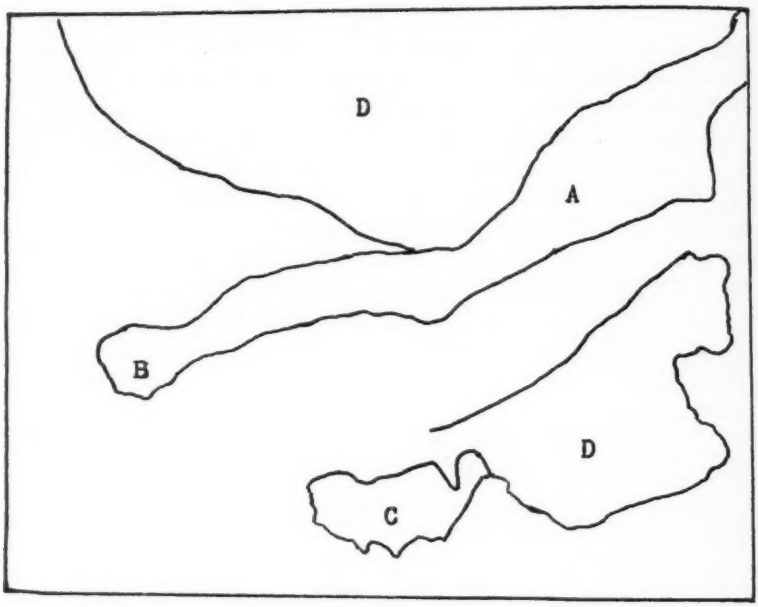


Fig. 1. Sicklemia spleen, not perfused. The follicular capillary entering the marginal zone at A terminates in the pulp cord at B. An empty venous sinusoid is shown at C. The "pools" of packed lamellated sickled erythrocytes are shown at D. Hematoxylin and eosin stain. × 950.

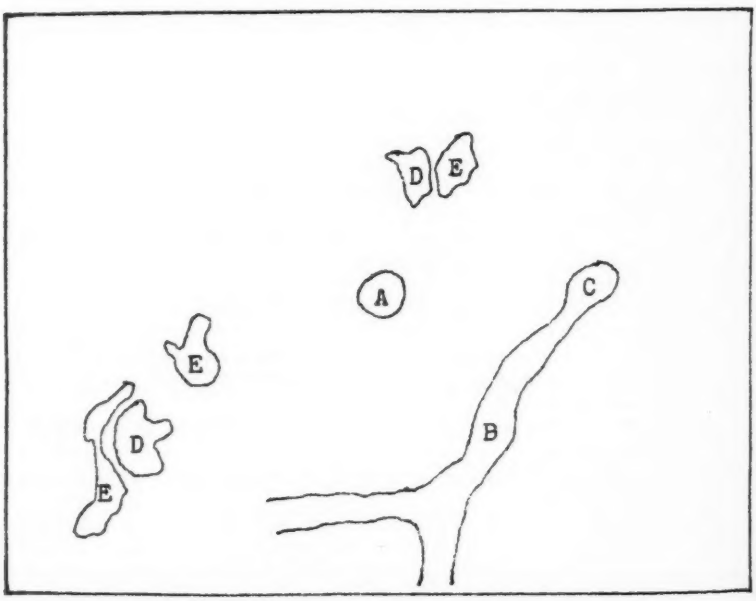
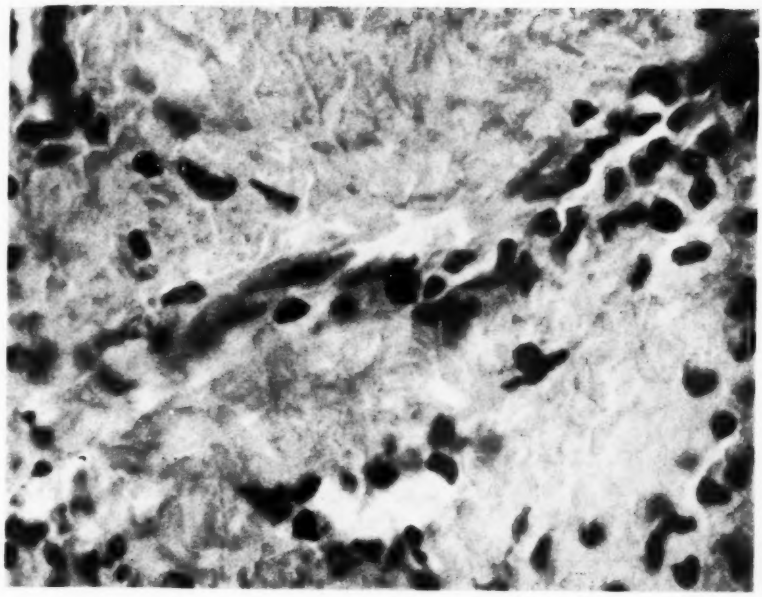


Fig. 2. Sicklemia spleen, arterially perfused. Central artery at A, marginal capillary, B, terminating in pulp cord at C. "Pools" in pulp cords are shown at D and empty venous sinuses at E. Hematoxylin and eosin stain. × 200.



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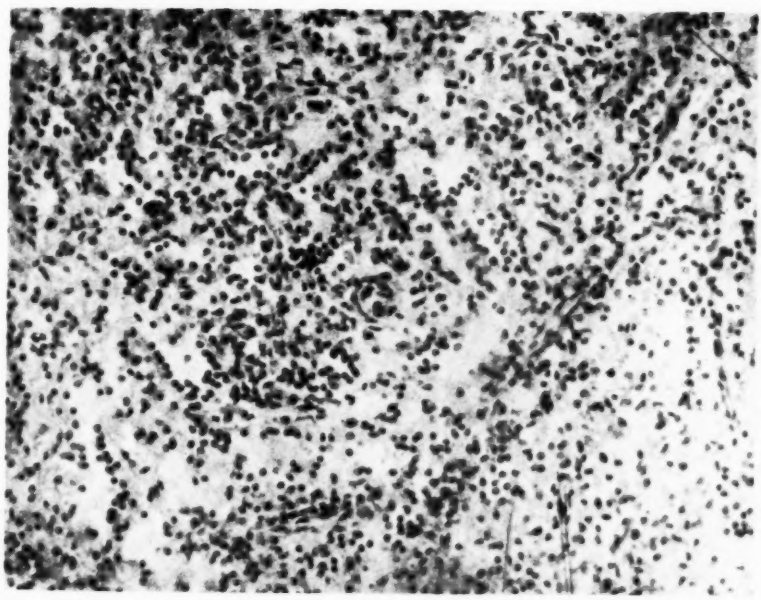

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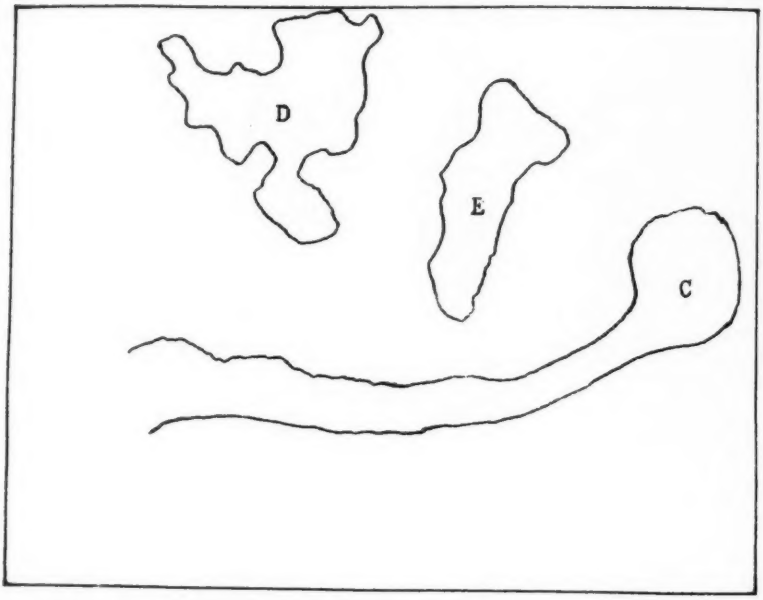


Fig. 3. Higher magnification of Figure 2 showing marginal capillary termination, C, with distention of pulp cords at D and empty venous sinusoids at E. Hematoxylin and eosin stain. × 950.

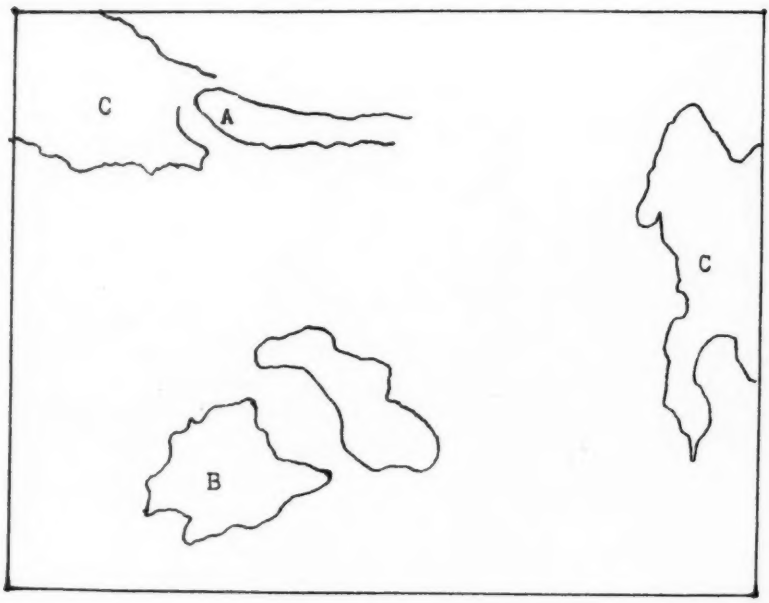
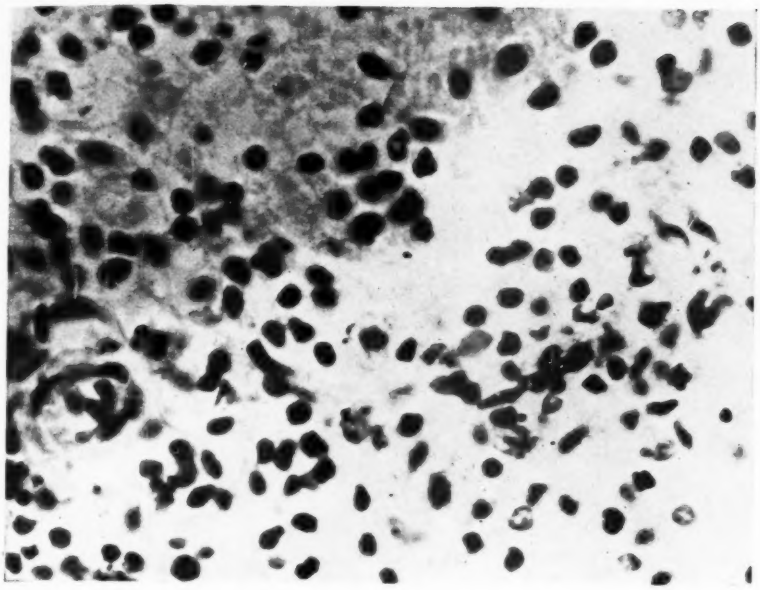


Fig. 4. Sicklemia spleen, perfused through veins. Follicular capillary termination in pulp cord at A, empty venous sinus at B. Distention of the pulp cords is shown at C. Hematoxylin and eosin stain. × 950.



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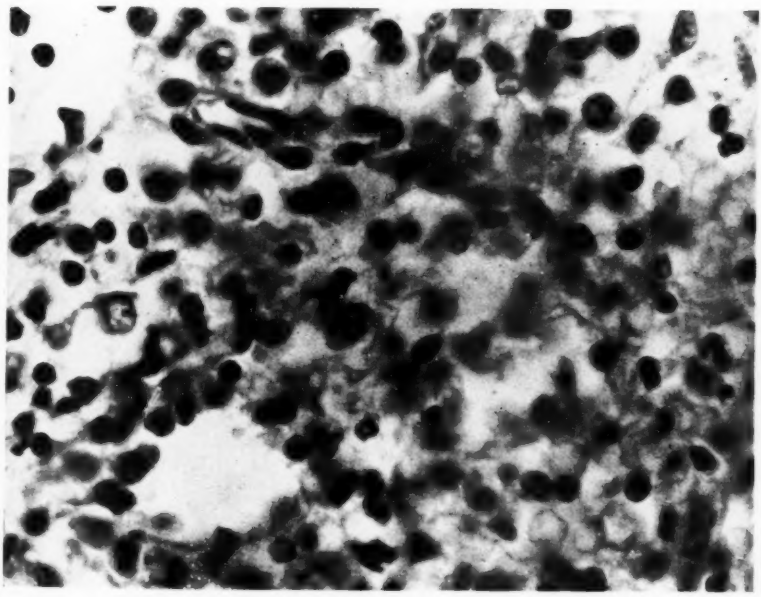

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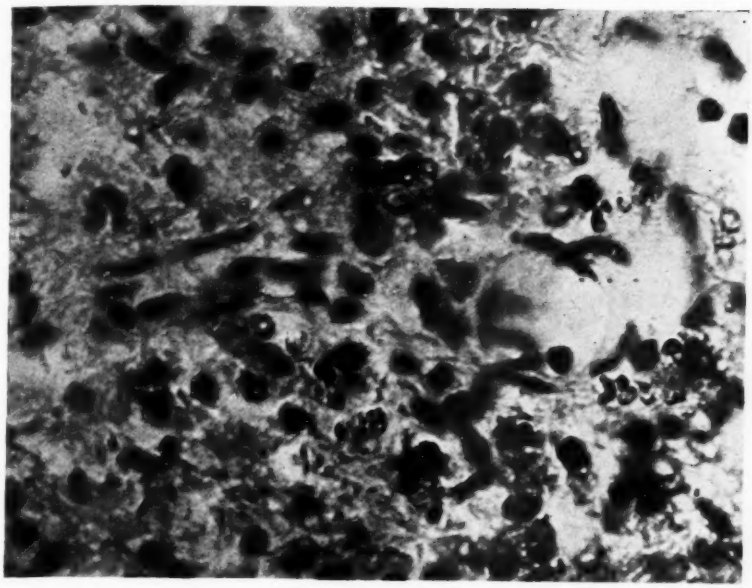
PLATE 148

FIG. 5. Sickle cell anemia spleen, perfused with distilled water and fixed in distended state. The follicular capillary crosses from left to right, terminating in a slight ampullatous dilatation in the pulp cord. Phagocytized pigment is contained in the reticulo-endothelial cells of the pulp cord. Hematoxylin and eosin stain. × 950.

FIG. 6. Sicklemia spleen, perfused with distilled water and fixed in distended state. The follicular capillary crosses from left to right, ending in an ampulla in the pulp cord. Cells of the malpighian corpuscles are seen in the left half of the field. Gomori's silver stain. × 1000.



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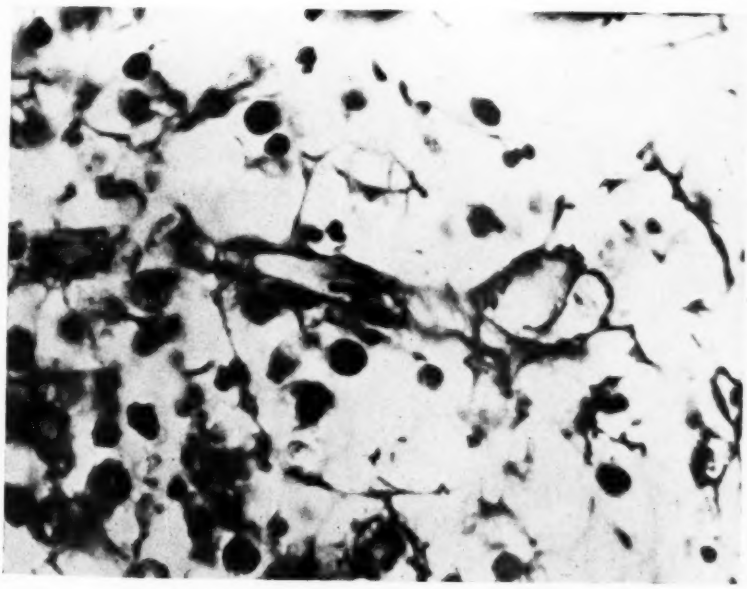

6

THE MICROSCOPIC DIAGNOSIS OF PULMONARY EMPHYSEMA *

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A still rather widely accepted concept of the nature of the pathologic changes in parenchymal pulmonary emphysema is that of rupture of the walls of contiguous alveoli with coalescence of their lumina into large single spaces. In support of this view, broken portions of alveolar septa terminating in bulbs formed by curling of the severed ends are described as projecting into the air spaces. From a comparison with sections of normal lungs preserved in the state of expansion such an interpretation seems to be erroneous. Although similar doubts concerning alveolar rupture in emphysema have been expressed in the literature, persistence of the idea in a number of standard works on pathology has stimulated this report. An attempt will be made to demonstrate that the significant pathologic changes in simple emphysema are increased size and altered form of the terminal air spaces of the lung. Only in emphysema complicated by formation of subpleural *blebs*,[1] pneumothorax, or pulmonary interstitial emphysema [2] does alveolar rupture occur.

Factors Influencing the Microscopic Appearance of the Lung

The gross and microscopic appearances of advanced stages of emphysema are so characteristic that little need be said about them. In early or doubtful cases naked-eye inspection may be of little help and the diagnosis may rest largely on microscopic examination. Many factors other than emphysema may influence the size and form of the terminal air spaces. Some of these will be discussed briefly.

The lung is a uniquely and exceedingly labile structure. Manipulations incidental to its removal, pressure exerted during palpation, and the effects of slicing the delicate tissue may cause striking changes in the microscopic appearance to a degree impossible in more stable organs such as liver, spleen, and kidney. The usual method of fixation employed at autopsy consists of excising a block of fresh lung tissue and placing it in fixative, commonly 10 per cent formalin or Zenker's solution. This technic, hereinafter referred to as fixation by immersion, does not consistently preserve the tissue at a uniform degree of expansion, for the size and form of the terminal air spaces in sections cut from blocks so treated vary greatly. Manipulation may greatly compress the tissue in some regions and correspondingly distend it in more

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peripheral zones by forcing air, edema fluid, or blood into the alveolar spaces and capillaries. Normal lung collapses when the thorax is opened and the usual picture in immersion-fixed material is that of nonexpansion. Air forced into the subpleural air spaces by proximally exerted pressure or by pathologic alveolar contents such as pneumonic exudates may produce a microscopic picture of expansion and even hyperexpansion in place of the usual collapse. Chronically overdistended lung tissue with diminished elasticity fails to collapse and in immersion-fixed sections also commonly appears expanded, but as emphysema is only one of several factors which can produce this picture, such expansion *per se* cannot be accepted as diagnostic of emphysema. It thus becomes apparent that it is desirable to preserve the lung in some manner which will produce a uniform state of expansion, eliminating extraneous factors so that the distention of emphysema may be accorded greater significance. A useful method is that of introducing fixative into the isolated lungs through the trachea until they have attained a size equal to that of normal inspiration. No matter what method of fixation is adopted, it is necessary to distinguish between the microscopic appearance of expansion in normal and in emphysematous lungs.

The Terminal Air Spaces in Expanded Normal Lungs (Three-Dimensional Consideration)

The anatomy of the terminal air spaces of the lung has been the subject of extensive investigations by Macklin,[3] Miller,[4] Willson,[5] and others. Only a brief account of those aspects considered most pertinent to an understanding of the changes occurring in emphysema is included here.

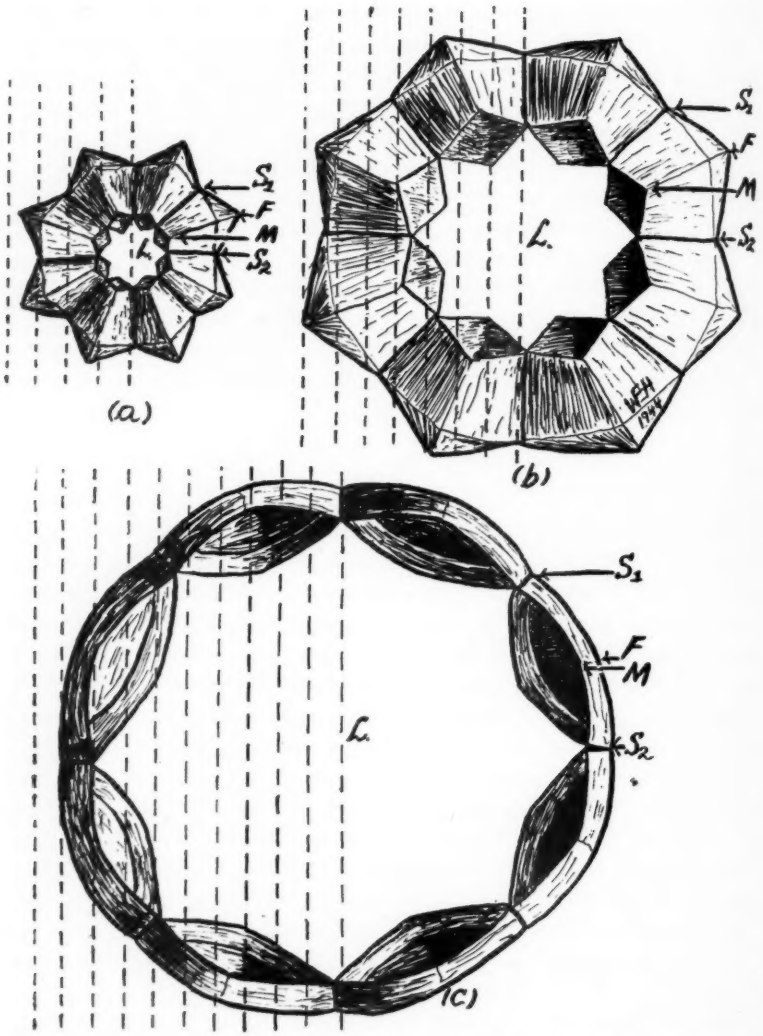
Those subdivisions of the lung subject to significant alterations in emphysema are included in the phrase *terminal air spaces* herein used to refer to alveolar ducts, alveolar sacs, and alveoli. From respiratory bronchioles several *alveolar ducts* arise with walls formed almost entirely by the openings of smaller, blind outpouchings or bays which surround them on all sides (Text-Fig. 1 and Figure 1). These are the *alveoli*. Each alveolar duct ends in one or more slightly expanded terminations, the *alveolar sacs*, similarly surrounded on all aspects by the mouths of alveoli which form their walls. Unless an alveolar duct is cut longitudinally, it cannot be distinguished in microscopic sections from an alveolar sac, for the cross section of a duct has all the appearances of a sac except for a slightly smaller diameter, a difference not sufficiently great for identification. For simplicity the term, alveolar ducts, will be used to refer to both ducts and sacs, for distinction between these two is not relevant to the diagnosis of emphysema. It is,

however, essential that the distinction between alveolar *ducts* and their encircling *alveoli* be clearly envisioned in comparing the terminal air spaces in sections of expanded and collapsed lung. This distinction may be emphasized by comparing an alveolar duct to the central passage of a barn in which rows of open stalls lining both sides represent alveoli. For completeness one must visualize the ceiling and floor as also lined by other open stalls, and the central passage should not be rectangular but approximately circular.

Each alveolus possesses a relatively wide mouth surrounded by a sphincter-like, fine band of smooth muscle,[3] and side walls which slope gently outwards to a base slightly larger than the mouth. The alveolar bases which intervene between neighboring ducts are most frequently in the form of cones, the central point being located most distant from the alveolar mouth.

The Appearance of the Terminal Air Spaces in Sections of Normal Lung Preserved in the State of Expansion (Two-Dimensional Appearance)

Figure 1 is from a section of nonemphysematous, adult human lung filled with Bouin's picric-formol solution introduced under moderate pressure into the bronchial tree through a tracheal cannula until the lung attained a degree of expansion comparable to that of normal inspiration. A portion of an alveolar duct (which terminates in an alveolar sac) is illustrated, and the manner in which both are bounded by alveoli is shown. The latter appear as *open* U-shaped outlines, each separated from its neighbor by a common, inwardly projecting septum which ends in a small bulge or knob representing a cross section of the muscle ring which encircles each alveolar mouth. This appearance of an alveolar duct is the result of being sectioned in a plane passing through the central lumen. If, instead, a tangential cut passes through only one side of the duct, the central lumen will not be included in the resulting section and only the row of alveoli forming that wall through which the knife passed will be seen. Since such a cut passes through the alveoli at an angle to their mouth-base axes, the alveolar mouths are "missed," and the alveoli appear in the section as uninterrupted, *closed*, irregularly circular outlines lying next to one another. These two types of alveolar outlines—*open* and *closed*—are not derived from differently constructed alveoli, but are the result of sectioning a single type in different directions. *Zig-zag* lines [6] are another feature to be noted in sections of expanded normal lung. They are formed wherever a series of sectioned conical bases separating alveoli of neighboring alveolar ducts are seen. Each base appears V-shaped in outline and dovetails with others in a manner somewhat suggesting the cells of a honeycomb. A row of alternating V's produces a *zig-zag* line.



Text-Fig. 1. This is a diagrammatic representation of eight alveoli surrounding the lumen (L.) of an alveolar duct in cross section at various stages of expansion. For the sake of simplicity the alveoli are represented as four-sided whereas actually they are probably more often octahedral. The dotted, parallel, vertical lines represent a series of random sections passing through each three-dimensional figure.

(a) Depicts the alveoli in a partial degree of collapse. The alveolar dimension from mouth to conical base is greater than width or diameter taken at right angles to the mouth-base depth. In the series of sections indicated by the dotted lines only one would pass through the lumen of the alveolar ducts and by cutting the alveolar mouths produce *open* alveolar outlines.

(b) Depicts the alveoli in a state of expansion comparable to normal inspiration.

The alveolar depth and width, as defined above, are nearly equal. The sections indicated by the dotted lines would include the lumen of the alveolar ducts in about half the cases and by passing through the alveolar mouths *open* alveolar outlines would be produced. In the remaining half the sections would contain only *closed* outlines.

(c) Represents the alveoli in emphysema. Alveolar diameter or width greatly exceeds depth as measured from mouth to base. Nearly all the sections indicated by the dotted lines would pass through the lumen of the alveolar ducts, and in only one would *closed* outlines be seen. By comparing (a) and (c), the manner in which the greater proportion of air-containing space changes from alveolar to alveolar ductal in nature may be seen.

In each of the diagrams, S_1 and S_2 indicate comparable positions on the alveolar septa; M, the alveolar mouth; and F, the floor or base. As the alveolar diameter indicated by the distance between S_1 and S_2 increases, reaching a maximum in emphysema (c), the alveolar depth measured between M and F decreases. The *ratio* of depth to alveolar circumference thus decreases as the terminal air spaces are progressively expanded.

The Terminal Air Spaces in Normal Lungs in the State of Collapse (Three-Dimensional Consideration)

In sections of lung obtained at autopsy the terminal air spaces are most commonly observed in varying degrees of collapse. As the air leaves the alveoli their side walls come closer together, the circumference diminishes, and depth as measured from mouth to base increases. Their shape thus changes from that of a cup to that of a test tube. Since the circumference of a duct is the sum of the diameters of the mouths of the alveoli forming its boundary in any one plane, this decrease in the diameter of a collapsed alveolus is accompanied by a corresponding diminution in the duct circumference. This is illustrated diagrammatically in Text-Fig. 1-a. The chief characteristics of a collapsed alveolus are thus increased depth and decreased circumference with a corresponding diminution in the size of the parent alveolar duct. Due to their elasticity, there is also an actual contraction of the alveolar walls and bases.

The Appearance of the Terminal Air Spaces in Sections of Normal Lung Preserved in the State of Collapse (Two-Dimensional Appearance)

Figure 2 is of a section of lung which has been preserved by immersion in fixative with no precaution to prevent collapse. The terminal air spaces are much smaller, but even more significant is the change in the alveolar proportions. In addition to an actual decrease in the capacity of the alveolar spaces, a change has occurred in the ratio of alveolar depth to circumference, the former increasing and the latter decreasing. As a result of the small size of the lumen of the alveolar duct (Text-Fig. 1-a), and the relative increase in alveolar depth, random sections do not pass through the alveolar mouths as often as in expanded lungs. *Open* alveolar outlines are thus relatively rare and *closed* outlines are correspondingly more frequent in sections of collapsed than in those of expanded lungs. The *zig-zag* lines formed by

the rows of contiguous alveolar bases in sections of collapsed lungs are prominent, presenting a serrated appearance.

The Terminal Air Spaces in Emphysematous Lungs (Three-Dimensional Consideration)

Emphysema is essentially a condition of hyperexpansion of the terminal air spaces. Increase in size and alteration of the form and proportions of the alveoli and alveolar ducts are the cardinal features (Text-Fig. 1-c). With this expansion there is an absolute increase in the area of the alveolar septa and bases produced by stretching the elastic membranes forming them. To facilitate a maximum increase in the alveolar circumference there is also a decrease in the alveolar depth as measured from mouth to base. The total area of the side walls of any alveolus is the product of circumference times depth, and as the former increases the latter must decrease in a manner comparable to the narrowing of a rubber band in the direction at right angles to that in which it is stretched. In an alveolar septum this change is made possible by virtue of its unattached free border surrounding the mouth which retracts towards the base, thus diminishing the actual depth as well as the ratio of depth to circumference, for the latter increases as depth decreases (Text-Fig. 1). Compared to the side walls the alveolar base is subject to greater stretching for it is attached at every border to the surrounding septa, but its expansion is facilitated by an almost complete loss of its conical form which becomes flattened. The lumina of the alveolar ducts are increased not only by the expansion of the alveolar mouths, which together represent the duct circumference in any one plane, but also by the decrease in alveolar depth which thus encroaches to a much less degree upon the central duct space. The results of these changes are an absolute increase in the size of the terminal air spaces and the conversion of a large proportion of alveolar space to parent duct space. It is important to note that, as described, this partial conversion of space from small alveolar subdivisions to larger alveolar ducts may occur without actual rupture of the alveolar septa. Of physiologic significance is the fact that in hyperexpansion a large proportion of the contained air is thus relatively distant from septal capillaries, whereas in normal expansion nearly all the air in the respiratory portion of the lung is in fairly close proximity to capillaries.

The Appearance of the Terminal Air Spaces in Sections of Emphysematous Lungs (Two-Dimensional Appearance)

In sections of emphysematous lung preserved routinely by immersion in fixative, the tissue nearly always appears in an expanded condition. Figure 3 is a photomicrograph illustrating emphysematous

lung preserved by the method used for that shown in Figure 1. As a result of the diminished alveolar depth, the alveolar septa project for only a short distance into the enlarged lumina of the alveolar ducts. The dimension, depth, taken approximately at right angles to the diminished mouth-base line, is increased as indicated by the enlarged circumference. The loss of the V-shapes of the alveolar bases incident to their stretching has smoothed out the *zig-zag* lines. Due to their shallow saucer-shape, few alveoli have been sectioned in planes which do not pass through their wide mouths and a high proportion of the resulting outlines are *open* rather than *closed*. The characteristic microscopic features of such sections thus include short alveolar septa, wide alveolar mouths, bases which join to form smooth rather than zig-zag lines, a predominance of open alveolar outlines over closed, and an inclusion by alveolar ducts of a large part of the space formerly bounded by alveoli. The increase in alveolar diameter taken at right angles to depth has produced an increase in the circumference of the alveolar ducts of which they form the walls.

The Microscopic Diagnosis of Emphysema

The characteristics described in the preceding section are easily observed in moderate degrees of emphysema. In sections prepared from advanced cases these features are exaggerated. In the latter instance the process of atrophy is added so that the inwardly projecting alveolar septa may be barely distinguishable or even absent, thus converting the lumina of the alveolar ducts into large, smooth-walled, irregularly circular spaces no longer lined by alveoli. Such an occurrence in the subpleural zone results in a *bulla*.[1]

In incipient or early cases the microscopic diagnosis is not always apparent, and here the need for specific criteria is evident. The greatest difficulty lies in distinguishing sections of only slightly emphysematous lungs from those in which emphysema is absent, but a degree of expansion has been preserved even though fixed by the immersion technic. In emphysema the lung tissue is in a permanent state of hyperexpansion, and collapse is not likely to occur even after death, but persistence of expansion in routine sections of lung, as shown earlier, is not pathognomonic of the condition. An example of non-emphysematous lung in which both a primarily compressed and secondarily distended area is included in the same section is illustrated in Figure 4. In this case the compression was purposefully produced but it demonstrates how such an appearance might be accidentally brought about, for this section is from tissue subsequently preserved in the usual manner by immersion in 10 per cent formalin.

Varying degrees of distention were produced in nonemphysematous lungs by digital compression, as above, and by injection of fixative into the bronchial tree under high pressure, expanding the tissue beyond normal inspiration. Microscopic examination failed to reveal a sufficient decrease in the alveolar mouth-base dimension and in the length of the inwardly projecting alveolar septa to be significant of true emphysema. Marked decrease of the septal height is apparently pathognomonic of emphysematous distention which has been present during life. It was found that even forceful manipulations will not produce this feature. Similarly, uniform obliteration of the *zig-zag* lines has not been produced by technical manipulation after death. These features—shortening of the interalveolar septa and obliteration of the *zig-zag* lines—are therefore offered as specific criteria for the microscopic diagnosis of emphysema.

Incipient Emphysema: a Special Problem

In the first stages of emphysema the condition may conceivably consist of only persistent expansion of the terminal air spaces which no longer undergo the usual degree of collapse during expansion. The change would at this stage be functional rather than anatomical, there being little or no alveolar structural alteration. It may well be argued that examination of the lung at such a stage would not exhibit the features described above, namely, shortening of the alveolar septa and flattening of the bases with obliteration of zig-zag lines. In these cases increased size of alveolar ducts and alveoli may be the only morphologic change. Quantitative estimations demonstrating a significant increase in average alveolar diameter over the normal would then constitute the only microscopic criterion upon which to base a diagnosis. Such estimations are too laborious and time-consuming for practical application. Methods involving preservation of the lung at carefully controlled degrees of expansion, and estimation of specific alveolar dimensions in sections 25 μ thick prepared therefrom, have been described [7] for the normal. In the absence of quantitative studies on specially prepared sections, I am unable to suggest other, more easily demonstrated features pathognomonic of emphysema in its early stages and sufficiently characteristic for diagnostic purposes. Further studies may provide new light on this problem. In this connection changes in the elastic tissue have been suggested and these will be briefly considered.

Elastic Tissue in Emphysema

It is generally realized that the elastic recoil of normal lung tissue is absent or diminished in emphysema. This is doubtless associated with the chronic state of hyperexpansion of the terminal air spaces which is

the characteristic feature of the condition. Whether this diminished elasticity produces the condition or is its result is outside the scope of this paper. In long-standing cases, elastic tissue cannot be demonstrated or is markedly reduced throughout the alveolar walls in the affected regions. Here, however, the altered size and proportions of the alveoli are so characteristic that no further features are necessary to establish the diagnosis. In earlier cases I have not observed any demonstrable decrease in the elastic fibers. In the diagnosis of early emphysema from microscopic sections, elastic tissue stains are thus of little practical aid.

Alveolar Rupture

If the evidence for alveolar rupture in emphysema, referred to in textbooks of pathology, be considered in the light of the microscopic appearance of expanded normal lung tissue described earlier, it is apparent that the so-called ruptured septa are merely the free ends of open alveolar outlines. The textbook figures illustrating broken portions of alveolar septa terminating in bulbs, thought to be formed by curling of the severed ends, are actually those of normal, inwardly projecting septa terminating in the bulge caused by cross sections of the smooth muscle surrounding the alveolar mouths.[8] Such alveolar septa constitute the side walls of *open* alveolar outlines. Many pathologists are familiar with sections of the normal lung only in a state of collapse. In such sections the majority of the alveoli appear as *closed* outlines, as previously explained, and the muscle bundles surrounding the mouths of any *open* outlines which may be present are not apparent, for the entire wall in the relaxed, collapsed stage is as thick as the terminal muscle bundle. Since *open* outlines are few in sections of collapsed lungs, the large number seen in expanded lungs might easily lend credence to the view that they represented *closed* outlines which had been ruptured and thus converted from the original O-shape to a U-shape. This is the result of considering the finer subdivisions of the lung only in a two-dimensional sense, as seen in sections, instead of correlating the appearances of these thin slices with that of the original three-dimensional alveoli and alveolar ducts. It is hoped that such correlation as has been attempted here will lead to the more general acceptance of shallow, wide alveoli and smoothing of the *zig-zag* lines formed by their bases as the microscopic criteria of emphysema, rather than alveolar rupture with coalescence of their lumina into single large spaces.

It is not denied that severe stretching of the alveoli may sometimes produce rupture, but the most likely site for occurrence is not the alveolar side walls but the alveolar bases, particularly those abutting against either the pleura or vascular sheaths. Pores (Macklin[9]) are

absent in such bases which are thus subjected to increased pressure only from one side. Rupture at these sites results in the formation of pleural *blebs* [1] and possibly pneumothorax, or, in the case of alveolar bases lying against vascular sheaths, produces pulmonary interstitial emphysema.[2] The emphysematous condition is then no longer uncomplicated, and such sequelae do not come within the scope of this paper.

Conclusions

1. Evidence has been presented to show that in uncomplicated emphysema rupture of the alveolar walls is rarely seen. Appearances suggestive of this in microscopic sections are in reality those of open alveolar outlines produced by sectioning the terminal expanded air spaces in planes passing through the mouths of the alveoli forming the walls of alveolar ducts.

2. Three microscopic features of emphysematous lungs are suggested as diagnostic criteria: (a) marked decrease in the average alveolar depth; (b) a corresponding increase in average alveolar diameter; (c) flattening of the alveolar bases.

3. Quantitative methods for estimating small increases in average alveolar diameter are suggested for the recognition of emphysema in early or doubtful cases.

4. If rupture occurs, the emphysematous condition is no longer uncomplicated. The most common site for rupture is at alveolar bases abutting against either the pleura or vascular sheaths. This may result in pleural *blebs* or pneumothorax in the first instance, and pulmonary interstitial emphysema in the second.

I wish to thank Professor William Boyd of the Department of Pathology, University of Toronto, for his criticism and aid in the preparation of this paper.

References

1. Miller, W. S. A further study of emphysematous blebs. *Am. J. Roentgenol.*, 1927, **18**, 42–47.

2. Macklin, C. C. The pattern of interstitial emphysema induced in the excised lung of the calf by overinflation. *Tr. Roy. Soc. Canada*, 1940, s. 3, **34** (Sect. V), 69–79.

3. Macklin, C. C. Functional aspects of bronchial muscle and elastic tissue. *Arch. Surg.*, 1929, **19**, 1212–1235.

4. Miller, W. S. The Lung. C. C. Thomas Co., Springfield, Ill., 1937.

5. Willson, H. G. The emphysematous lung. *Univ. of Toronto Med. Bull.*, 1927, **8**, 9–14.

6. Macklin, C. C. Personal communication, 1943.

7. Hartroft, W. S., and Macklin, C. C. Intrabronchial fixation of the human lung for purposes of alveolar measurement, using 25 μ microsections made therefrom. *Tr. Roy. Soc. Canada,* 1943, s. 3, **37** (Sect. V), 75–78. The size of human lung alveoli expressed as diameters of selected alveolar outlines as seen in specially prepared 25 μ microsections. *Ibid.,* 1944, s. 3, **38,** (Sect. V), 63.

8. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1938, ed. 3, pp. 453–454.

9. Macklin, C. C. Alveolar pores and their significance in the human lung. *Arch. Path.,* 1936, **21,** 202–216.

[*Illustrations follow*]

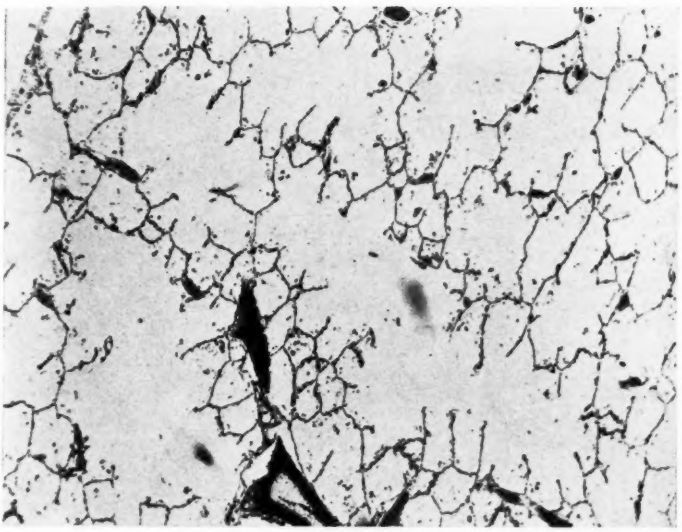
DESCRIPTION OF PLATES

PLATE 149

FIG. 1. Section of normal, adult human lung preserved in the state of expansion by intrabronchial filling with fixative. Open alveolar outlines and zig-zag lines between neighboring alveolar ducts are well shown. × 36.

FIG. 2. Normal, adult human lung preserved by immersion in fixative. The air spaces are partially collapsed. The small size of these alveoli necessitated a higher magnification than that used for Figure 1 in order that the alveolar form could be shown. The alveolus opening into the lower left corner of the alveolar duct is of test tube form. × 155.

1



2

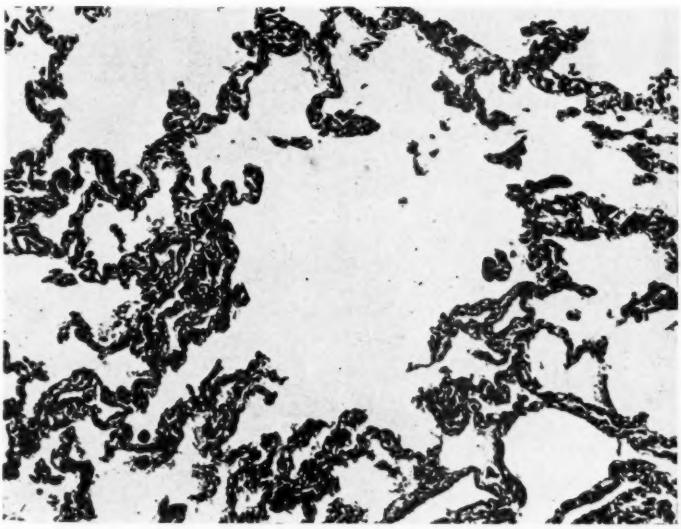


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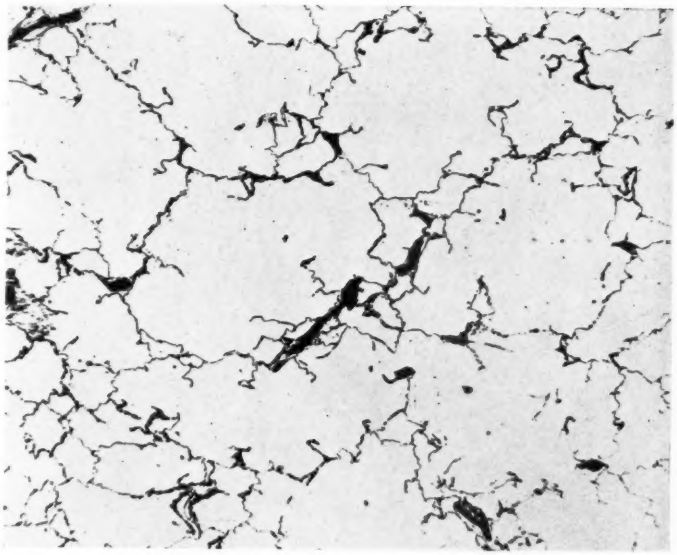
PLATE 150

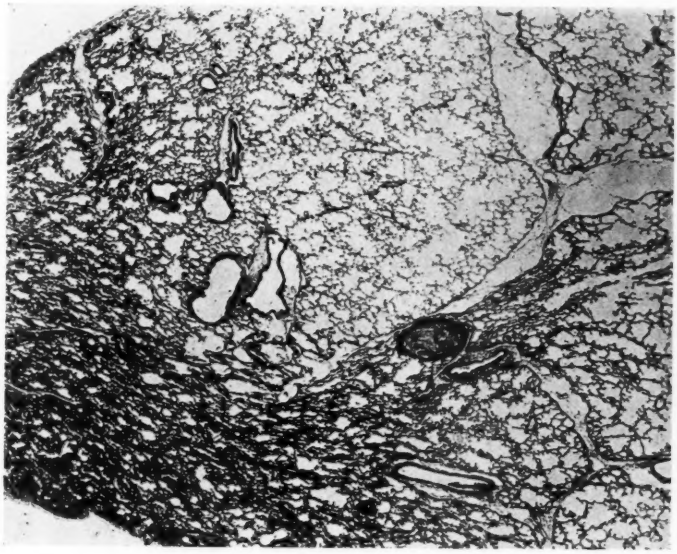
FIG. 3. Section of adult human, emphysematous lung preserved by the same method employed for the lung of Figure 1. As compared with Figure 1, increased breadth and shallowness of the alveoli and smoothing of the zig-zag lines are shown. × 36.

FIG. 4. Normal, adult human lung illustrating how manipulation may produce compression in one region and distention in adjoining peripheral portions of the tissue. Lung preserved by immersion in fixative. × 5.

3



4



Hartroft

Microscopic Diagnosis of Pulmonary Emphysema

THE VISCERAL LESIONS IN MEASLES

With a Report of Koplik Spots in the Colon *

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Although lesions of the lymphatic tissues of the abdomen in measles have attracted much attention, intestinal Koplik spots have been described but once. Hobson, who was unable to secure material for microscopic examination, observed them in a colostomy stoma. Others have commented on the possibility of a general intestinal response. Newman and Milstead noted bluish discoloration of the cecum during appendectomy in a case in which the lesions of measles were later demonstrated in the appendix. Stryker found Warthin-Finkeldey giant cells in Peyer's patches and Herzberg suggested that there might well be widespread lesions of the lymphoid tissue elsewhere than in the appendix.

The present report is of a fatal case of measles in which Koplik spots were conspicuous in the colon.

Report of Case

The patient, L. K., was a male child, 22 months old, who had been hospitalized in May, 1943, for acute influenzal meningitis, followed by moderate hydrocephalus, and again in November, 1943, for bronchopneumonia, from which he recovered uneventfully. The rest of his past history was not contributory, and he had never had measles as far as could be ascertained. Two days before the present admission, the child welfare doctor called at the foster home to see the patient's twin brother, who had measles with Koplik spots and a typical rash. L. K. had a slight cough and cold at that time, but no Koplik spots or rash. Two days later, April 19, 1944, he developed a measly rash and convulsions, and was brought to Grasslands Hospital. On admission, he was markedly cyanotic. The convulsions were stopped with sodium phenobarbital and vinethene.‡ He was given an oral airway and frequent aspirations were performed. In spite of these measures, he had difficulty in breathing, cyanosis and convulsions reappeared, and he died 5 hours after admission. The rash disappeared an hour or so before death.

Gross Examination

At post-mortem examination, performed 17 hours after death, a splotchy, cyanotic mottling of the skin was noted over the chest and face, but no evidence of a measles rash remained. Each pleural cavity contained a small amount of clear, straw-colored fluid. The lungs weighed 175 gm., and were slightly emphysematous, with moderate congestion at the bases. The cut section revealed a moist, bright sal-

* Received for publication, November 30, 1944.

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‡ A proprietary form of vinyl ether, phenyl alphanaphthylamine.

mon pink surface with slight mottling at the bases. The trachea and bronchi contained a small amount of yellowish white mucoid material.

The thymus was not unusual in size or appearance. The peribronchial and paratracheal lymph nodes were slightly enlarged.

The esophagus, stomach, duodenum, and rectum were not remarkable grossly. The Peyer's patches of the ileum were bright pink, sharply circumscribed, and hypertrophied. Extending from the base of the cecum to 6 inches below the ileocecal valve, the mucosa of the large bowel contained, in gradually decreasing number, many slightly elevated pink areas, 5 mm. or less in diameter. The center of each areola was marked by a slightly depressed, gray punctum of pin-point size. The intervening mucosa was not visibly inflamed, but the thickness of the intestinal wall was slightly increased by edema. The appendix appeared to be normal. The mesenteric lymph nodes were soft, from 0.5 to 1.5 cm. in diameter, and gave the mesentery a gray, nodular appearance. The cut section of these and the thoracic nodes showed a fairly homogeneous ivory white surface.

The heart, liver, spleen, kidneys, adrenals, and other organs were not remarkable except for various degrees of congestion. Only the spleen contained noteworthy microscopic lesions. Chronic adhesive arachnoiditis and hydrocephalus were present but they are not germane to this discussion. No signs of acute disease, other than vascular congestion, were noted in the central nervous system either grossly or microscopically.

Microscopic Examination

Microscopic examination of the lungs revealed a moderately severe interstitial pneumonia, with a mononuclear cell infiltration in the bronchial walls and surrounding alveoli, and an acute emphysema. The most interesting microscopic changes were seen in the epithelium of the bronchi and in the peribronchial lymphatic tissue.

The epithelial change was a peculiar fusion and exfoliation of wide sheets of epithelium into the lumina of the bronchi and bronchioles. The cilia were preserved, but the nuclei of the epithelial cells were gathered into central grape-like masses, showing hyperchromatism, pyknosis, and karyorrhexis (Fig. 1). The cell boundaries were indistinguishable and the cytoplasm was homogeneous and strongly eosinophilic. The denuded bronchial walls showed regeneration of epithelium, and in some areas, where sloughing was not marked, squamous metaplasia (Fig. 2). These changes involved the bronchial tree to the terminal bronchioles, and, rarely, these fused epithelial sheets were found loose in the alveoli. In addition, the lumina of the bronchioles and bronchi contained a small amount of exudate consisting of leukocytes, fibrin, and large vacuolated mononuclear round cells.

The other change was the formation of giant cells of the Warthin-Finkeldey type in the peribronchial lymphoid tissue (Fig. 2). These were not numerous, but each section of lung contained a few. The giant cells were mulberry shaped, with 5 to 20 dark-staining nuclei grouped in a mass in the center or to one side of the cell. The cytoplasm was homogeneous and eosinophilic, with a definite, rounded border. Various stains were used to demonstrate inclusion bodies in the lung and elsewhere, but without success.

The thymus was somewhat hyperplastic. Relatively few Hassall's bodies were present. The most remarkable feature was a multitude of giant cells, so numerous, dense, and hyperchromatic as first to suggest artifacts. They were of the Warthin-Finkeldey type, but rather small and with scanty cytoplasm (Fig. 4). They occurred in fairly distinct lacunae throughout the gland and were most numerous in the cortex of the lobules.

The spleen and lymph nodes of the abdomen and thorax presented a similar appearance. Occasional Warthin-Finkeldey giant cells were seen, tending to occur near the periphery of the secondary nodules in each organ. The secondary nodules also showed central "lymphoid exhaustion" (decrease of lymphoid elements, leaving a rather barren reticulum) and peripheral vascular congestion. The form of the giant cells was similar to that observed in the other organs but the cytoplasm was more abundant than in the thymic giant cells.

The Peyer's patches of the ileum were markedly enlarged, and surrounded by plexuses of distended veins. The center of each secondary nodule showed lymphoid exhaustion, and toward the periphery many contained one or more giant cells. The loose connective tissue adjoining the lymphoid follicles was infiltrated with lymphocytes, plasma cells, tissue eosinophils, and mast cells. The epithelium was thinned and occasionally eroded. Large numbers of giant cells of the specific measles type were found adjacent to the lymphoid nodules, but few within them. They occurred principally in the substantia propria of the villi and none were seen below the mucosa. In general, they were slightly larger than the other giant cells observed, but showed wide variation in size and shape (Fig. 3). The appendix contained analogous lesions, with an exudate in the lumen consisting of small round cells, fibrin, and cellular débris. The walls of both the appendix and ileum were slightly edematous.

The Koplik spots in the colon resembled the lesions of the ileum. Each proved to be an enlarged lymphoid follicle showing central exhaustion, containing a few giant cells, and surrounded at its periphery and in the loose connective tissue of the immediate mucosa by distended vessels. The villi adjacent to the follicles were crowded with giant cells

resembling those in the ileum, and there was similar infiltration with lymphocytes, plasma cells, and mast cells. The edema was slightly more marked than in the ileum (Figs. 5 and 6).

Discussion

Warthin-Finkeldey Giant Cells

The reaction of tissues, other than cutaneous, to the virus of measles has provoked much interest since the simultaneous and independent reports of Warthin and Finkeldey, in 1931, of the presence of giant cells in the tonsils in the prodromal stage of measles. Similar changes had been found in tissues studied by Ciaccio and Alagna in 1910 and 1911 respectively. Alagna, who limited his study to the mucous membrane of the nose and throat, found large multinucleated giant cells in the tonsils, and Ciaccio, observing tissues from the same cases, saw cells resembling megakaryocytes in the newly formed capillaries in the interstitial tissue of the lungs. He also noted swelling of the intestinal lymphoid tissue, but saw no giant cells.

Since Warthin's and Finkeldey's reports, this peculiar, mulberry shaped, syncytial giant cell has been observed in many tissues, and apparently only in association with measles. There has been one report of its presence in the tonsils of a child believed to have chicken pox, but the evidence excluding measles as the diagnosis is rather tenuous.

The relation of Warthin-Finkeldey giant cells to measles has become so widely accepted that Gordon and Knighton used their presence in lymphoid tissue as the criterion of the disease in their studies of the measles virus. In a well controlled experiment, four monkeys were inoculated with blood taken from human patients within 24 hours after the appearance of the measles rash. Lymph nodes were removed from the monkeys on alternate days, and in three monkeys giant cells were found in constant but diminishing numbers from the third to the tenth day after inoculation. None of the animals had clinical signs of measles. The photomicrographs accompanying the report leave little doubt that the giant cells seen were typical. This work also confirms observations in human cases which indicate that giant cells are a manifestation of the prodromal and early acute phases of the disease.

Table I shows the widespread distribution of the Warthin-Finkeldey cells in cases of human measles and will serve as a brief outline of the literature concerning their location and time of appearance. Except as indicated in footnotes to the table, the diagnosis usually was based on the presence of Koplik spots in the mouth, as well as on the systemic signs and an exanthem.

The origin of the specific Warthin-Finkeldey giant cell has been considered by Mulligan, who concluded that they are formed "as a result of the polynuclear abnormal development of the stem cell paralleling the mononuclear normal development of the lymphocyte from the stem cell." Other workers believe them to originate from amitotic division

TABLE I

Reported Distribution of the Giant Cells of Measles and Correlation to the Stage of the Disease

Author	Cases	Location of giant cells	Stage of disease
1. Ciaccio, 1910, and Alagna, 1911	8	Tonsil	Not specified
2. Warthin, 1931	1	Tonsil	4 days before rash
3. Finkeldey, 1931	1	Tonsil	3 days before rash
1932	1	Appendix	2 days before rash
4. Herzberg, 1932	1	Appendix	4 days before rash
5. Davidsohn and Mora, 1932	1	Appendix	3 days before rash
	1	Appendix	2 days before rash
	1	Appendix (rare)	2 days after rash
6. Fischer, 1933	1	Appendix	1½ days before rash
7. Schultze, 1933	1	Appendix	2 days before rash
8. Hathaway, 1935*	1	Spleen, abdominal lymph nodes	Exposed
9. Bullowa, *et al.*, 1937	3	Appendix	Pre-eruptive
10. Gräff, 1937	1	Tonsils, lymph nodes, fossa of Rosenmüller	15 days after exposure
11. Wegelin, 1937	1	Appendix	4 days before rash
	1	Appendix	3 days before rash
	1	Appendix	Day rash appeared
12. Semsroth, 1939†	1	Tonsil, spleen, thymus, lymph nodes, tongue, and bronchi	Exposed
13. Stryker, 1940‡	1	Lungs, tracheal and bronchial mucous glands, lymph nodes, spleen, and Peyer's patches	Exposed
14. Newman and Milstead, 1940	1	Appendix	3 days before rash
15. Mulligan, 1944	1	Appendix	3 days before rash

* This patient was exposed by two older siblings and died without the appearance of a rash or Koplik spots.

† This patient was exposed by two other siblings and died 12 days after the rash appeared in one child, on the day it appeared on the other child. No rash or Koplik spots were present in the case in question.

‡ This patient was exposed in a nursery, presumably.

of plasma cells or lymphocytes, from mesenchymal or reticulum cells, or from fusion of lymphocytes. The last seems the most likely from consideration of our slides, although the large number of plasma cells found in the tissue surrounding the giant cells leads one to suspect that they might be implicated in giant cell formation.

Fused Epithelial Giant Cells

Although there is an almost universal tendency to discount the giant cell of the fused respiratory epithelium type, emphasized by Masugi and Minami, I believe it is as important in the study of this disease as

the syncytial Warthin-Finkeldey giant cell. In some preparations of lung tissues from cases of measles, in which the architecture was destroyed by inflammatory changes, it is impossible to distinguish between the two. Also, occasionally, the epithelium in the intestinal tract shows similar fusion. As far as may be determined in a study of fixed tissues, the transitional stages involved in the formation of both epithelial and lymphoid giant cells, followed by karyorrhexis and pyknosis of their nuclei, are identical. It is probably this kind of cellular response, rather than specific tissue susceptibility, which is characteristic of measles.

MacCallum noted the presence of fused epithelial masses in the lung in his study of pneumonia in army camps during the last war, where measles was the most common predisposing disease, and Stryker also mentioned them. Denton, in 1925, was struck by their appearance and wrote:

> "In many bronchioles the wall cannot be made out, for it often shades off into swollen, contiguous alveolar walls. In places, alveoli close to bronchial radicles contain large mononuclear cells from the respiratory epithelium and fused sheets of atypical respiratory epithelium. Numerous giant cells with five to twenty nuclei and well preserved or degenerating cell bodies are present. . . . Dark-staining compact masses of polylobate nuclei, between which no cell boundaries can be seen, lie in some alveolar spaces."

These epithelial giant cells formed a very conspicuous feature in the present case, but were confined principally to the bronchi and bronchioles. Hecht was able to produce extensive and similar lesions by the intratracheal administration of metallic salts and ammonia, which vitiates any conclusions regarding the specificity of these cells for measles, but does not prevent one from suspecting that they may arise from a similar alteration in the cell membrane, whatever the responsible agent may be.

The relationship between the epithelial giant cell and the giant cell of "giant cell pneumonia" in infants and children merits observation. The latter is considered a rare disease most often occurring after measles, and characterized histologically by the presence of giant cells of the Langhans type in the alveoli. Moore and Gross, who considered them to be the result of fusion and hyalinization of exudates, distinguished between them and the fused bronchial epithelial giant cell. Chown believed the giant cell of pneumonia to be the result of metaplasia of alveolar epithelium under the influence of vitamin A deficiency. Whatever the derivation of the cells, they bear more than a superficial resemblance to the bronchial epithelial giant cells which I observed, and may represent a more severe response to the same agent or agents. Although they were found both with and without squamous metaplasia in my case, the possible rôle of vitamin A deficiency acting with a virus may be considered in a study of their pathogenesis. Dietary informa-

tion in my patient is lacking, but the child previously had been admitted to the hospital with evidence of malnutrition.

Inclusion Bodies

In undertaking the report of this case, opportunity to compare the histologic sections with those from his previously reported series was afforded by Dr. James Denton. Similar lesions were noted, varying only

TABLE II

Inclusion Bodies in Measles

Author	System or cell	Type of inclusion	Staining reaction
1. Ewing, 1909	Epithelium of skin and mucous membranes, sweat glands, endothelium	Cytoplasmic, granular, and ring forms in perinuclear vacuoles	Basophilic
2. Mallory and Medlar, 1919	Endothelium of Koplik spots and cutaneous rash	Cytoplasmic, spherical (believed to be cocci)	Basophilic
3. Farber and Wolbach, 1932*	Salivary glands	a. Nuclear, ovoid b. Cytoplasmic	Acidophilic Basophilic
4. Masugi and Minami, 1938	Fused bronchial epithelium, acini of sublingual and salivary glands, buccal mucous glands	Cytoplasmic, round	Eosinophilic
5. Broadhurst, *et al.*, 1937	a. Smears of Koplik spots and nasal mucosa: in basal and columnar cells and lymphocytes	Nuclear (originally)	Nigrosin-staining
1938	b. Blood and tissue cultures: in leukocytes, lymphocytes and fibroblasts	Nuclear (?), granular	Basophilic
6. Semsroth, 1939	a. Giant cells in epithelium of tongue	Cytoplasmic, globoid	Basophilic
	b. Giant cells in tonsil	Cytoplasmic, globoid	Eosinophilic
	c. Fused bronchial epithelial giant cells	Cytoplasmic, globoid	Eosinophilic
7. Goodpasture, *et al.*, 1939†	Epithelium of bronchi, trachea, bronchial mucous glands, and alveolar epithelium	Nuclear, compact but granular	Slightly acidophilic

* Farber and Wolbach observed inclusions in the salivary glands of many infants dying of various causes, one of whom had had measles 4 months previously.

† Goodpasture and others studied five cases of virus pneumonia in children, three of whom had measles. The inclusions were similar in all five cases.

in degree. The principal interest in studying his slides was directed toward inclusion bodies, since none could be found in my material. Before describing my findings, I should like to present a table, which is by no means exhaustive, giving the observations of various workers in this connection (Table II).

Rivers considered the characteristic inclusion body of measles to be cytoplasmic, but attached to it little diagnostic importance.

In three of Denton's cases, I have been able to find eosinophilic,

cytoplasmic inclusion bodies. They were present in the bronchial epithelium (Fig. 7), in fused bronchial epithelial giant cells, in bronchial mucous glands, and in submucous glands involved in the Koplik spots. Inclusions were not found in the above locations in all cases, but each had them in cells of one or more types. The bodies were usually round, homogeneous, of variable size, multiple, and occurred anywhere in the cytoplasm, but generally toward the free edge of the cell. As can be seen in Table II, Semsroth, and Masugi and Minami have reported inclusions of the same type. It is necessary to agree with Rivers, however, that their presence is far from diagnostic of measles, for I have observed similar forms in various epithelia in canine distemper (Green and Evans), and in the nasal mucosa of monkeys with lymphocytic choriomeningitis.

Similar cytoplasmic inclusions have been found in the bronchial epithelium, and zona reticularis and medulla of the adrenal glands of infants dying of "primary virus pneumonitis," as reported by Adams. They differ in being surrounded by a halo, and by appearing singly in the cell. The commonest precursors of this type of pneumonia, according to Adams, are "the common cold, grip, influenza, and measles." The blood of the affected infants in Adams' series was tested only for neutralizing antibodies of influenza virus, and none were found. It would be interesting to know how many of the infants surviving the epidemic subsequently developed measles.

Summary

1. A case of fatal measles is presented in which Koplik spots were found in the colon, associated with large numbers of Warthin-Finkeldey giant cells in the intestine and other organs.

2. The specificity of these giant cells for measles is accepted and the evidence for their widespread occurrence in the tissues of patients in prodromal and initial stages of measles is reviewed.

3. The fused epithelial giant cells found in this case and reported by others are believed to be as important in the study of measles as the syncytial Warthin-Finkeldey giant cells, although probably they are not specific for measles. The giant cells of "giant cell pneumonia" of infants may represent a similar response to the same agent or agents.

4. Search for inclusion bodies gave conflicting results in the material available for study. Similar conflicting observations have been reported by various observers.

I wish to thank Dr. Gilbert Dalldorf and Dr. George Y. McClure for advice and assistance in the preparation of this paper, and Dr. James Denton for the use of his histologic sections of measles material.

BIBLIOGRAPHY

Adams, J. A. Primary virus pneumonitis with cytoplasmic inclusion bodies. *J. A. M. A.*, 1941, **116**, 925–933.

Alagna, G. Histopathologische Veränderungen der Tonsille und der Schleimhaut der ersten Luftwege bei Masern. *Arch. f. Laryngol. u. Rhinol.*, 1911, **25**, 527–530.

Broadhurst, J., MacLean, M. E., and Saurino, V. Inclusion bodies in measles. *J. Infect. Dis.*, 1937, **61**, 201–207.

Broadhurst, J., Cameron, G., and Saurino, V. Measles inclusion bodies in blood and in tissue cultures. *J. Infect. Dis.*, 1938, **62**, 6–20.

Bullowa, J. G. M., McCabe, E. J., and Wishik, S. M. Acute appendicitis in the exanthems. *Am. J. Dis. Child.*, 1937, **53**, 1029–1038.

Chown, B. Giant cell pneumonia of infancy as a manifestation of vitamin A deficiency. *Am. J. Dis. Child.*, 1939, **57**, 489–505.

Ciaccio, C. Beitrag zur pathologischen Anatomie und zur Mikrobiologie der Masern. *Virchows Arch. f. path. Anat.*, 1910, **199**, 378–400.

Dalldorf, G. Personal communication.

Davidsohn, I., and Mora, J. M. Appendicitis in measles. *Arch. Path.*, 1932, **14**, 757–765.

Denton, J. The pathology of fatal measles. *Am. J. M. Sc.*, 1925, **169**, 531–543.

Ewing, J. The epithelial cell changes in measles. *J. Infect. Dis.*, 1909, **6**, 1–16.

Farber, S., and Wolbach, S. B. Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. *Am. J. Path.*, 1932, **8**, 123–135.

Finkeldey, W. Über Riesenzellbefunde in den Gaumenmandeln, zugleich ein Beitrag zur Histopathologie der Mandelveränderungen im Maserninkubationsstadium. *Virchows Arch. f. path. Anat.*, 1931, **281**, 323–329.

Finkeldey, W. Riesenzellbefunde bei akuter Wurmfortsatzentzündung. Ein Beitrag zur Histopathologie der Veränderungen des Wurmfortsatzes im Maserninkubationsstadium. *Virchows Arch. f. path. Anat.*, 1932, **284**, 518–525.

Fischer, W. Über die Diagnose der Masern im Prodromalstadium. Eigenartige Befunde am lymphatischen Apparat der Appendix. *Beitr. z. path. Anat. u. z. allg. Path.*, 1933, **91**, 474–482.

Goodpasture, E. W., Auerbach, S. H., Swanson, H. S., and Cotter, E. F. Virus pneumonia of infants secondary to epidemic infections. *Am. J. Dis. Child.*, 1939, **57**, 997–1011.

Gordon, H., and Knighton, H. T. Experimental measles. The lymphoid tissues of animals inoculated with the virus of human measles. *Am. J. Path.*, 1941, **17**, 165–176.

Gräff, S. Primärinfekt und Primärkomplex der Masern. *Deutsche med. Wchnschr.*, 1937, **63**, 1357–1360.

Green, R. G., and Evans, C. A. A comparative study of distemper inclusions. *Am. J. Hyg.*, 1939, **29** (Sect. B), 73–87.

Hathaway, B. M. Generalized dissemination of giant cells in lymphoid tissue in prodromal stage of measles. *Arch. Path.*, 1935, **19**, 819–824.

Hecht, V. Die Riesenzellenpneumonie im Kindesalter. *Beitr. z. path. Anat. u. z. allg. Path.*, 1910, **48**, 263–310.

Herzberg, M. Giant cells in the lymphoid tissue of the appendix in the prodromal stage of measles. *J. A. M. A.*, 1932, **98**, 139–140.

Hobson, F. G. Koplik spots in the colon. *Lancet*, 1940, **2**, 134–135.

MacCallum, W. G. The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917–1918. Rockefeller Institute for Medical Research, New York, 1919, Monograph 10.

Mallory, F. B., and Medlar, E. M. The skin lesion in measles. *J. M. Research,* 1919–20, **41**, 327–348.

Masugi, M., and Minami, G. Über einen Fall von Masern mit Riesenzellenbildungen an Luftwegen, Mund- und Rachenschleimhaut. Über die Einschlüsse an Masernriesenzellen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1938, **101**, 483–502.

Moore, R. A., and Gross, P. Giant cells in inflammations of the lungs in children. *Am. J. Dis. Child.*, 1930, **40**, 247–259.

Mulligan, R. M. Genesis of the multinucleated giant cells in the lymphatic tissue of the appendix in measles. *Arch. Path.*, 1944, **37**, 61–67.

Newman, P. F., and Milstead, L. C. Appendical changes in the prodromal stage of measles. *J. Internat. Coll. Surgeons,* 1940, **3**, 551–555.

Rivers, T. M. Some general aspects of pathological conditions caused by filterable viruses. *Am. J. Path.*, 1928, **4**, 91–124.

Schultze, W. H. Die Wurmfortsatz im Prodromalstadium der Masern. *München. med. Wchnschr.*, 1933, **80**, 576–577.

Semsroth, K. H. Multinucleate epithelial giant cells with inclusion bodies in prodromal measles. Report of an autopsy. *Arch. Path.*, 1939, **28**, 386–389.

Stryker, W. A. Disseminated giant cell reaction: a possible prodrome of measles. *Am. J. Dis. Child.*, 1940, **59**, 468–478.

Warthin, A. S. Occurrence of numerous large giant cells in the tonsils and pharyngeal mucosa in the prodromal stage of measles. *Arch. Path.*, 1931, **11**, 864–874.

Wegelin, C. Zur histologischen Diagnose der Masern. *Schweiz. med. Wchnschr.*, 1937, **67**, 1–2.

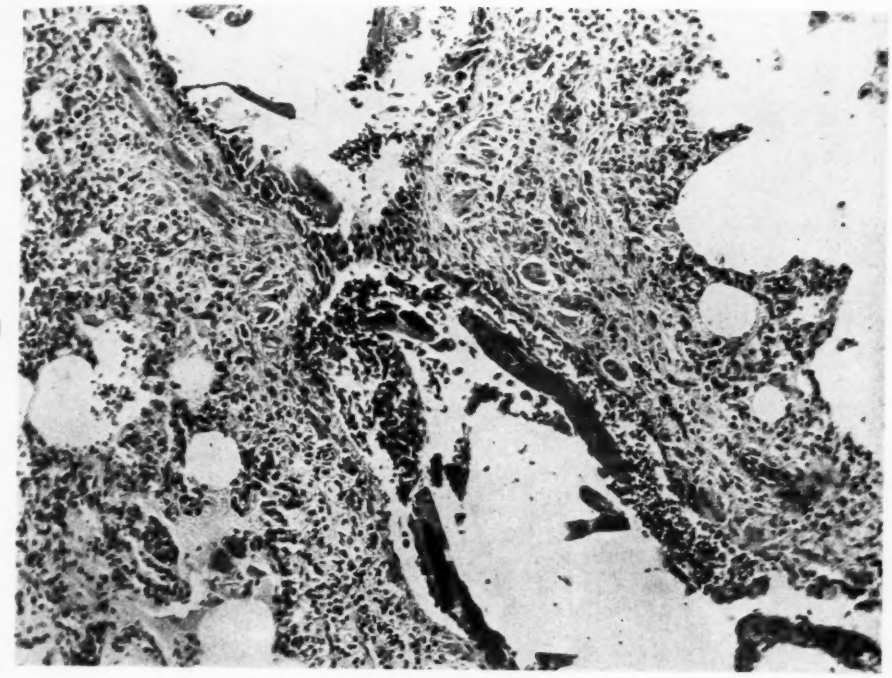
DESCRIPTION OF PLATES

PLATE 151

FIG. 1. Bronchus containing fused epithelial giant cells. × 200.

FIG. 2. Bronchus showing squamous metaplasia of the epithelium. A few rather small Warthin-Finkeldey giant cells may be seen in the lymphoid collection to the left. × 307. The inset shows a giant cell from an adjoining field, at a slightly higher magnification. Several basophilic granules were present in the cytoplasm, and one small eosinophilic inclusion was seen in the upper right-hand corner of the cell. (Denton's material.)

1



2

Corbett

Visceral Lesions in Measles

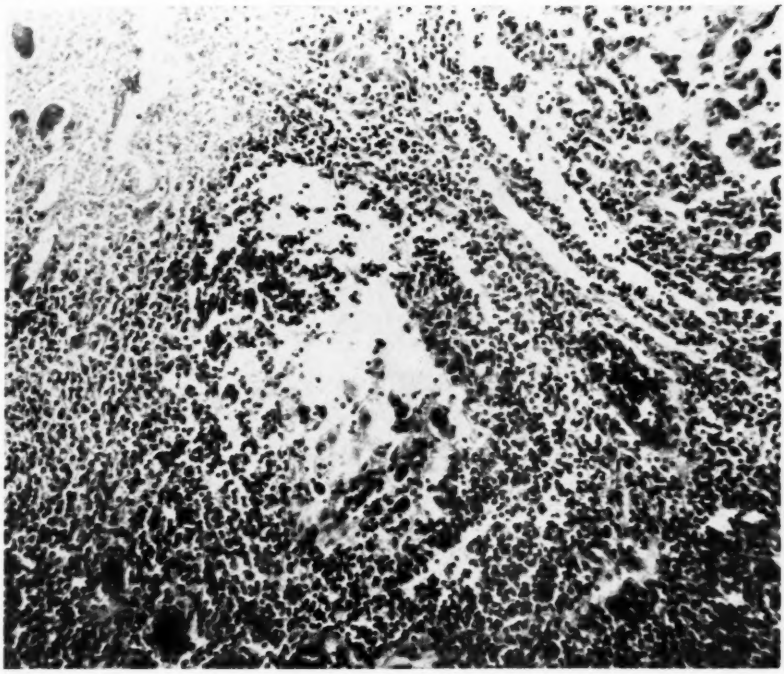
PLATE 152

FIG. 3. Peyer's patch in the ileum, showing necrosis of the epithelium (upper left), central lymphoid exhaustion, and four giant cells. × 307.

FIG. 4. Giant cells in the thymus. × 200.

FIG. 5. Mucosa of the colon adjacent to a Koplik spot, containing giant cells in the substantia propria. × 307.

3



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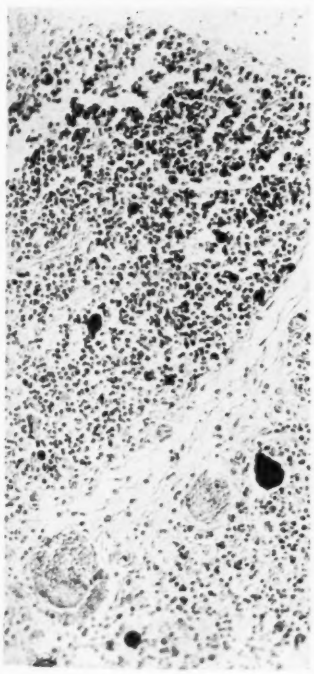

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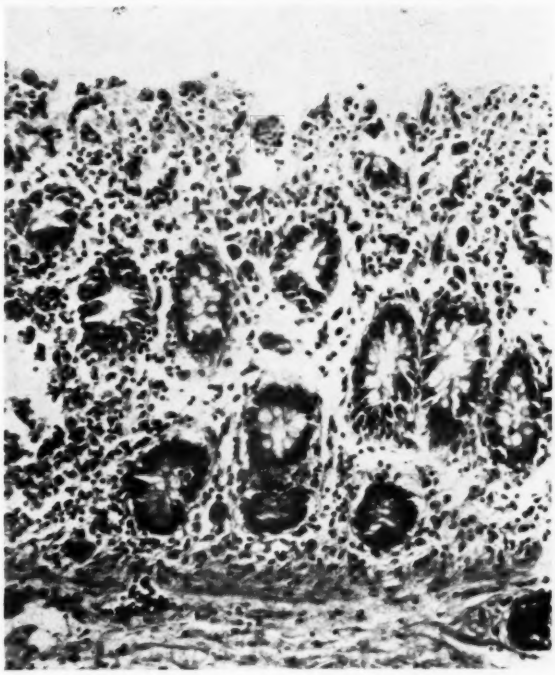

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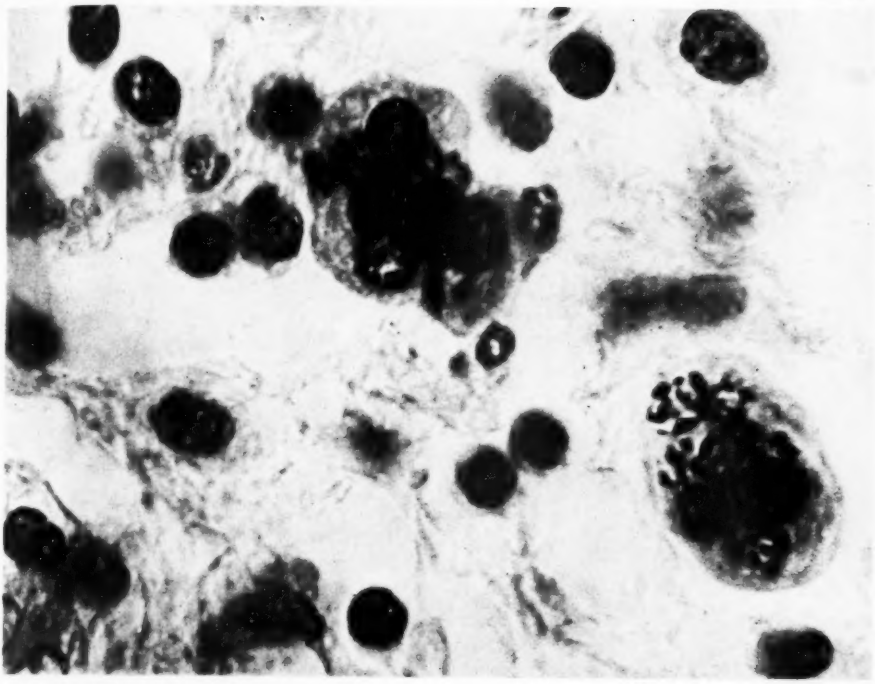
PLATE 153

FIG. 6. Detail of giant cells in the intestine. × 1820.

FIG. 7. Partially detached epithelial cell from a bronchus, containing four inclusion bodies. × 1820. (Denton's material.)

6



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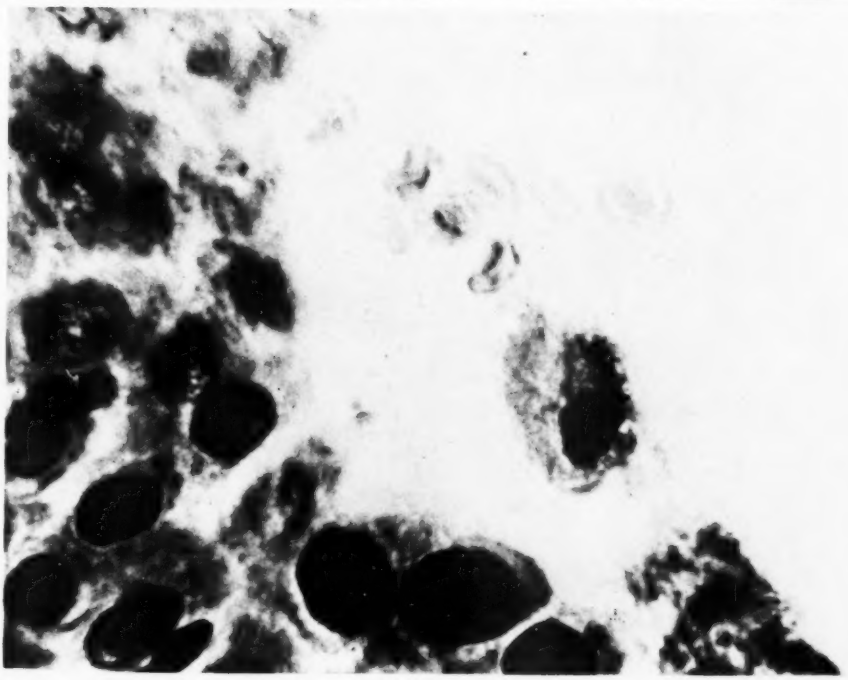

Corbett Visceral Lesions in Measles

UNIV. OF MICH.

EXTRAGENITAL CHORIOCARCINOMA IN THE MALE *

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Choriocarcinoma of the male is usually primary in the testis. Only rarely does it originate in other situations. Two possibilities exist as to extragenital origin, namely, from elements of teratomas or from embryonal rests of the urogenital fold. Dickson [1] and others have contended that they come from totipotent cells capable of forming trophoblastic tissue which can produce various structures including fetal chorionic tissue. Blastomeres may become dissociated wherever germinal epithelium is found. Arey [2] explained that due to limitation of lateral expansion by the dorsal body wall, the enlargement of the growing mesonephric tubules necessitates longitudinal growth on each side of the dorsal mesentery. Thus the urogenital fold ultimately extends from the sixth cervical to the second sacral segment. The ridge becomes divided into a lateral mesonephric fold and a median genital fold, the anlage of the genital gland. Hence there is a distinct possibility that the primary focus of a choriocarcinoma may be located at the hilum of the lung as a remnant of the urogenital fold persisting in the region of the thoracic segments.

Symeonidis,[3] Prym,[4] and others have emphasized the importance of careful examination of the testes in cases of choriocarcinoma supposedly of extragenital origin. Symeonidis was skeptical of extragenital origin in any case, referred to possible accessory abdominal testes as a source, and maintained that even if derived from a teratoma the tumor originates in trophoblastic tissue.

In Prym's [4] case there were lesions distributed as are metastases of testicular tumors and with a microscopic picture like choriocarcinoma. Because he found a scar, peculiar in its structure and vascularization, in the right testis, he assumed that a primary choriocarcinoma in that testis had undergone spontaneous retrogression. It is said that uterine choriocarcinoma may regress, but in such instances the original diagnosis may have been incorrect. Even if this is true, Prym had no proof that the testicular scar was due to healing of a neoplasm. Nevertheless, these observations indicate the importance of most careful examination of the testes for either tumor or scar in all cases thought to have originated in a situation other than testis. This includes complete study of the genital tract, with serial block sections of the testes.

Adherence to these rigid criteria excludes many cases reported as

* Received for publication, September 25, 1944.

extragenital choriocarcinomas. Among these is that of Bonn and Evans.[5] In this case the primary site was uncertain because a small scar was demonstrated in one testis. Although this scar may possibly have resulted from regression of a tumor, it seems more likely to have been due to an injury sustained 20 years before when the patient was kicked by a horse. Other probable cases * of primary extragenital choriocarcinoma, excluded because of uncertainty about the thoroughness of the examination of the testes, are those reported by Ritchie,[6] Lambert and Knox,[7] Hammarskjöld,[8] Arendt,[9] and Becker.[10] In all of these there were mediastinal teratomas and choriocarcinomatous metastases.

The number of cases, accepted as authentic, varies in different reports. If, however, strict criteria are adhered to and negative serial block sections of the testes considered essential, only 7 of the cases recorded in the literature furnished conclusive proof of an extragenital origin. To these is added the case reported in this paper, making a total of 8 unquestionably proved cases of primary extragenital choriocarcinoma in the male.*

1. Krassnianskaya.[11] Age 72 years. Primary either at hilum of left lung or in retroperitoneal tissue. A pure choriocarcinoma with multiple metastases. No gynecomastia. Testes showed no microscopic change. Aschheim-Zondek test not done.

2. Kantrowitz.[12] Age 22 years. Primary in the superior mediastinum. A complex teratoma in the superior mediastinum with choriocarcinomatous metastases in the lungs. No gynecomastia. Testes showed interstitial cell hyperplasia. Aschheim-Zondek test positive.

3. Fenster.[13] Age 27 years. Primary in retroperitoneal tissue. Pure choriocarcinoma with metastases to lungs and liver. Bilateral gynecomastia. Testes were normal. Aschheim-Zondek test not done.

4. Gerber.[14] Age 23 years. Primary in retroperitoneal tissue, overlying right ileopsoas muscle. Pure choriocarcinoma with metastases to lungs, liver, spleen, and kidneys. No gynecomastia. Testes showed no microscopic change. Aschheim-Zondek test not done.

5. Weinberg.[15] Age 70 years. Primary in urinary bladder. A pure choriocarcinoma with metastases to most of the viscera. No gynecomastia noted. Marked interstitial cell hyperplasia and tubular atrophy in the testes. Aschheim-Zondek test positive.

6. Erdmann, Brown, and Shaw.[16] Age 45 years. Primary in retroperitoneal tissue, posterior to left kidney. Pure choriocarcinoma with metastases to lungs. Enlargement of breasts, considered to be fibroadenomas, probably gynecomastia. Aschheim-Zondek test negative.

7. Hyman and Leiter.[17] Age 57 years. Pure choriocarcinoma, primary in urinary bladder. Bilateral orchiectomy performed. No autopsy. Testes showed no abnormality. Bilateral gynecomastia. Aschheim-Zondek test positive.

8. Laipply and Shipley. Age 21 years. Primary site was a complex teratoma of the superior mediastinum. Choriocarcinomatous metastases in many organs. Testes

* Since this paper was submitted for publication, Plenge[20] has reported a probable case, primary in the retroperitoneal region, and Stowell *et al.*[21] have described a well established case, primary in the region of the pineal gland.

showed marked atrophy, tubular fibrosis, and interstitial cell hyperplasia. Bilateral gynecomastia present. Aschheim-Zondek test positive.

In the above indisputable cases the primary sites were mediastinum (2), urinary bladder (2), retroperitoneal (3), and in 1 case either in the hilum of the lung or in the retroperitoneal tissue. In all instances the disease was fatal. The duration of life after onset of symptoms varied from 2 weeks to 15 months (average 5½ months).

Secondary changes in the breasts and testes are not uncommon in cases of choriocarcinoma. The enlargement of the breasts is usually bilateral and may be the first sign associated with such a tumor. The increase in size results from proliferation of ducts and stroma (Fig. 5). Definite gynecomastia was noted in 3 of the above 8 accepted cases, was doubtful in 1, and absent in 4. In the doubtful case [16] a diagnosis of adenofibroma was made on one breast which was removed prior to the patient's death. It seems possible that gynecomastia could have been mistaken for such a tumor.

The testicular changes include atrophy of the entire organ, fibrosis and hyalinization of the seminiferous tubules, and hyperplasia of the interstitial cells of Leydig (Figs. 6 and 7). Such features were noted in 7 of the 8 accepted cases. Similar changes have been observed in primary teratomas of the testes and in many other conditions such as senility, tuberculosis, cryptorchidism, and hermaphroditism. Houghton [18] described similar testicular alterations in a case of well differentiated malignant mediastinal teratoma.

Report of Case *

A white male, 13 years old, was first admitted to Mount Sinai Hospital, Cleveland, Ohio, in January, 1936. For 8 months he had had pain, intermittent in character, in the region of the left nipple. He was well nourished and normally developed. There was diffuse pulsation of the entire upper one-half of the thorax. Tactile fremitus and resonance were diminished over the upper third of the left lung. A tumor was located and removed from the superior mediastinum (Fig. 1). It extended laterally between the lobes of the left lung and was adherent posteriorly to the pericardium. Gross examination of the specimen revealed an irregularly nodular mass which weighed 385 gm. and measured 13 by 12 by 8 cm. Except in one area, 1 cm. in diameter, the mass was completely encapsulated. The bulk of the tumor was solid, with a few irregularly distributed cysts containing gelatinous and grumous material. Microscopic examination revealed it to be a complex teratoma. There were no trophoblastic elements and there was no evidence of malignant change. The following structures were identified: skin, hair, glands (sebaceous, serous, mucous, and salivary), mucous membrane (like that of upper respiratory tract and colon), brain, ganglia, nerves, smooth and skeletal muscle, fibrous connective tissue, fat, cancellous bone, and cartilage.

The patient recovered and was apparently well until July, 1942, 6½ years after

* We are indebted to Dr. S. O. Freedlander for permission to publish this case and to Dr. B. S. Kline for making available the gross specimen, microscopic sections, and photograph (Fig. 1) of the surgical specimen.

the operation. At this time a routine roentgenogram of the chest, taken on examination for entrance into the Navy, revealed a mediastinal tumor with multiple metastases to the lungs. In October, 1942, he was given 30 deep x-ray treatments to the lungs. The metastases increased in size during the treatments. In January, 1943, the patient was admitted to Lakeside Hospital. At this time physical examination revealed an emaciated white male, 21 years old. His temperature was 38°C.; pulse, 105; respirations, 24; blood pressure, 115/65 mm. of Hg. The breasts were increased in size, firm, and nodular. A spherical, firm nodule, 2 cm. in diameter, was present in the subcutaneous tissue of the right upper quadrant of the abdomen. The testes, which were present in the scrotum, were abnormally small. Secondary sex characteristics were well developed.

Laboratory Examination. Urine: trace of albumin, an occasional white blood cell and granular cast. Blood: 16,500 white blood cells, 2.07 million red blood cells, 36 per cent hemoglobin (Sahli), negative Kline exclusion test. Hormone assays; results given in Table I.

Soon after hospitalization the patient's condition was recognized as hopeless. Testosterone propionate (25 mg. per day) was given for 1 week in a futile attempt to depress the growth of the tumor. Because of the insistence of relatives he was given several blood transfusions. On his 11th hospital day the patient developed signs of bronchopneumonia. His temperature, which had varied between 36.7° and 38°C., rose to 39.5°C. Sulfadiazine was given without effect. He died on his 17th hospital day, 7 years after the teratoma was removed from the mediastinum.

Autopsy (no. 8014, performed by Dr. J. C. Sherrick) revealed a circumscribed tumor in the superior mediastinum and metastatic tumors in subcutaneous tissues of anterior abdominal and posterior thoracic walls, left deltoid muscle, lungs, right parietal pleura, diaphragm, liver, kidneys, jejunum, ileum, thoracic and abdominal lymph nodes, and greater omentum. Other important diagnoses were gynecomastia, fibrosis and interstitial cell hyperplasia of testes, and bronchopneumonia.

The mediastinal tumor (Fig. 2) was slightly to the left of the midline. It was spherical in shape, completely encapsulated, and measured 6 cm. in diameter. It was in large part solid but contained scattered cysts filled with translucent, yellow, gelatinous material. Near the periphery there were a few small, soft, hemorrhagic foci. Microscopically its complex nature was indicated by the presence of skin, fat, fibrous connective tissue, smooth muscle, hyaline cartilage, cancellous bone, salivary gland, ganglia, and mucosa, submucosa, and muscularis of bronchus and stomach. In addition, there were cell masses having the structure of a choriocarcinoma. Syncytia and cells of Langhans' type, which normally make up the trophoblastic covering of the chorionic villi, were present (Figs. 3 and 4). Such groups of cells could be located macroscopically by associated hemorrhage and necrosis. The metastatic tumor was similar at all sites. The nodules varied from 1 to 3 cm. in diameter, were sharply circumscribed, and in most instances dark red. Microscopically they contained syncytial and Langhans' cells. The red discoloration was due to recent hemorrhage. Small foci of necrosis were common.

Microscopic examination of the breasts revealed proliferation of ducts and stroma. The ducts were lined with stratified columnar epithelium. The periductal connective tissue was loosely arranged and sparsely infiltrated with lymphocytes (Fig. 5).

In order to exclude a primary testicular tumor, serial sections of the testes were made. The sections were cut at 6 μ and every tenth section was mounted. No tumor or scar was identified. This examination, therefore, excluded any lesion in the testes greater than 0.06 mm. in diameter. Both organs were small, weighing 7 and 6 gm. There was no spermatogenesis. The seminiferous tubules were small and hyalinized. The interstitial cells were markedly increased in numbers (Figs. 6 and 7).

Comment

During life the presence of gynecomastia and small testes suggested to the clinician the possibility of a feminizing tumor. For this reason hormone assays of the urine were done. The results were striking and

Table I

Hormone Assays on 24-Hour Urine Specimens

	Gonadotropin (chorionic type) in mouse units per 24 hrs.	Estrogen in international units per 24 hrs.	17 ketosteroid mg. per 24 hrs.	Pregnanediol mg. per 24 hrs.
Present case	300	750 or more	27	2 or more
Normal male	0	75 or less	25	0.1
Normal nonpregnant female	0	50 to 300 (ovulation)	12	45 (in luteal phase)
Teratoma of testis	Elevated	Not known	Not changed	Not known
Late pregnancy	6000	10,000	Not changed	30 to 40
Choriocarcinoma of uterus	200,000 to 1,000,000	Elevated	Not changed	Not known

probably diagnostic of choriocarcinoma. A comparison of the hormonal findings in this case with those in normal persons and related conditions is given in Table I.

In this case there is no way of determining whether the mediastinal tumor present at autopsy represents the growth of a small portion of the original teratoma left at the operation 7 years prior to death or the growth of another tumor of similar and subsequent origin.

The presence of chorionic gonadotropin in the urine along with increased excretion of estrogens and of pregnanediol is similar to the hormonal alteration which occurs in pregnancy. It may be presumed that these hormones or their precursors were elaborated by the chorionic tissue of the tumor.

Atrophy of the testicular tubules and gynecomastia were undoubtedly due to the large amount of circulating estrogen. A contributory stimulus to the enlargement of the breast may have been added by progesterone.

No exact quantitative method was used to determine the number of Leydig cells in the testes. It was estimated, however, that these cells were increased in number. The examination of a large number of sections of the testes to some extent decreases the error inherent in such an estimation. An actual increase in the number of testicular interstitial cells could be due either to direct stimulation by chorionic gonadotropin or to increased luteinizing hormone secreted by the anterior lobe of the pituitary gland in response to the high level of circulating estrogen. The work of Collins [19] indicates that Leydig cell hyperplasia may occur in cases without carcinoma and in the absence of known excess of luteinizing hormone or estrogen. From his work it is also apparent that hyperplasia of the interstitial testicular cells is associated with atrophy and fibrosis of the tubules but that tubular fibrosis and atrophy may occur without a significant change in the number of Leydig cells.

Summary

Review of the literature discloses only seven well established cases of extragenital choriocarcinoma in males. In the additional case reported in this paper a complex teratoma originating in the thorax of a boy, 13 years old, was removed surgically. Seven years later he died of either a recurrence or an independent teratoma with choriocarcinoma in the tumor and widespread choriocarcinomatous metastases. Gynecomastia, testicular atrophy, and hyperplasia of the interstitial cells of Leydig were associated. Hormonal alterations resembled those of pregnancy.

REFERENCES

1. Dickson, J. D. Chorioepithelioma: Should serum from the female in the puerperium and pregnancy be given a therapeutic trial? *U.S. Nav. M. Bull.*, 1935, **33**, 358–362.
2. Arey, L. B. Developmental Anatomy. W. B. Saunders Co., Philadelphia, 1931, ed. 2.
3. Symeonidis, A. Zur Frage der extragenitalen teratogenen Chorionepitheliome und der chorionepitheliomähnlichen Geschwülste. *Centralbl. f. allg. Path. u. path. Anat.*, 1935, **62**, 177–186.
4. Prym, P. Spontanheilung eines bösartigen, wahrscheinlich chorionepitheliomatösen Gewachses im Hoden. *Virchows Arch. f. path. Anat.*, 1927, **265**, 239–258.
5. Bonn, H. K., and Evans, N. Extragenital chorioepithelioma in the male with associated gynecomastia; report of a case. *Am. J. Surg.*, 1942, **58**, 125–132.
6. Ritchie, J. A case of embryoma occurring in the mediastinum. *J. Obst. & Gynaec. Brit. Emp.*, 1903, **4**, 65–73.

7. Lambert, S. W., and Knox, L. C. Intrathoracic teratoma. *Tr. A. Am. Physicians*, 1920, **35**, 17–62.
8. Hammarskjöld, B. A contribution to the knowledge of teratomas and dermoids in the anterior mediastinum. *Acta radiol.*, 1934, **15**, 210–224.
9. Arendt, J. Das Chorionepitheliom des Mannes. *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1931, **43**, 728–735.
10. Becker, B. J. P. Teratomata of the anterior mediastinum. (A review of their features with a report of an unusual case.) *South African M. J.*, 1939, **13**, 659–664.
11. Krassnianskaya, P. V. Causes of chorionepithelioma in men outside of sexual sphere. *Mosk. med. j.*, 1929, **9**, (no. 5), 1–7.
12. Kantrowitz, A. R. Extragenital chorionepithelioma in a male. *Am. J. Path.*, 1934, **10**, 531–543.
13. Fenster, E. Über ein extragenitales Chorionepitheliom beim Manne mit positiver Hypophysenvorderlappenreaktion. *Frankfurt. Ztschr. f. Path.*, 1934, **46**, 403–409.
14. Gerber, I. E. Ectopic chorioepithelioma. *J. Mt. Sinai Hosp.*, 1935, **2**, 135–142.
15. Weinberg, T. Primary chorionepithelioma of the urinary bladder in a male. *Am. J. Path.*, 1939, **15**, 783–795.
16. Erdmann, J. F., Brown, H. A., and Shaw, H. W. Chorioepithelioma in the male of extragenital origin. *Urol. & Cutan. Rev.*, 1941, **45**, 1–6.
17. Hyman, A., and Leiter, H. E. Extratesticular chorioepithelioma in a male, probably primary in the urinary bladder. *J. Mt. Sinai Hosp.*, 1943, **10**, 212–219.
18. Houghton, J. D. Malignant teratoma of mediastinum. Report of a case and review of 24 cases from the literature. *Am. J. Path.*, 1936, **12**, 349–371.
19. Collins, E. E. Somatic carcinoma and the state of the interstitial cells of the testicle. *Arch. Path.*, 1936, **22**, 470–476.
20. Plenge, K. Zur Frage des extragenitalen Chorionepithelioms beim Mann. *Virchows Arch. f. path. Anat.*, 1944, **312**, 643–651.
21. Stowell, R. E., Sachs, E., and Russell, W. O. Primary intracranial chorionepithelioma with metastases to the lungs. *Am. J. Path.*, 1945, **21**, 787–801.

[*Illustrations follow*]

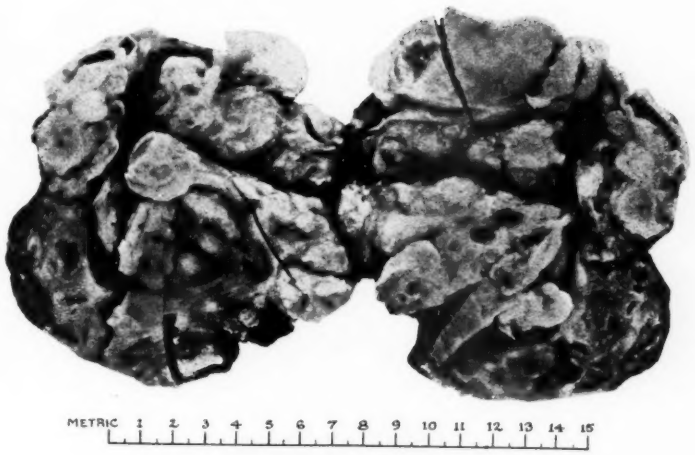
DESCRIPTION OF PLATES

PLATE 154

FIG. 1. Tumor removed from upper mediastinum, 7 years before patient's death. This was a complex benign teratoma. The metric scale which is reproduced is for comparison with Figure 1 only.

FIG. 2. Sections of lungs with mediastinal teratoma attached to medial aspect of upper lobe of left lung. Multiple hemorrhagic metastatic choriocarcinomatous nodules in both lungs.

1



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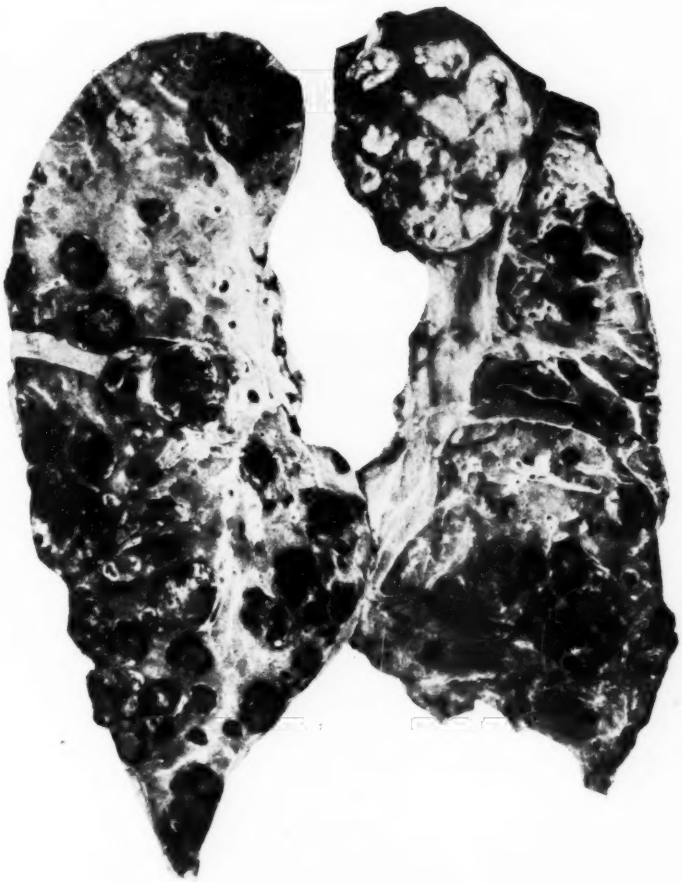
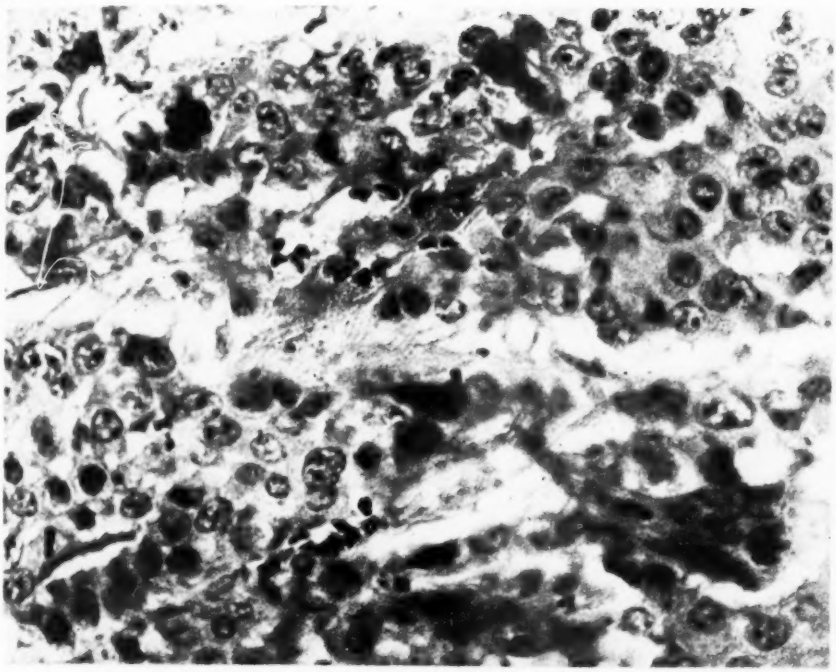


PLATE 155

FIG. 3. Pulmonary metastasis with syncytia, many cells of Langhans' type, and mitotic figures. Hematoxylin and eosin stain. × 538.

FIG. 4. Pulmonary metastasis. Discrete syncytia and groups of Langhans' cells are evident. Hematoxylin and eosin stain. × 582.

3



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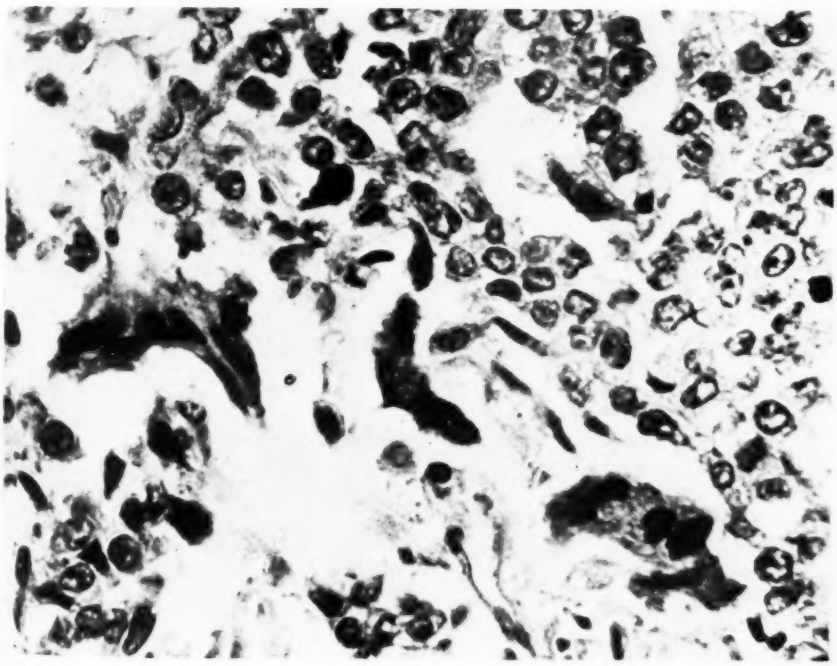


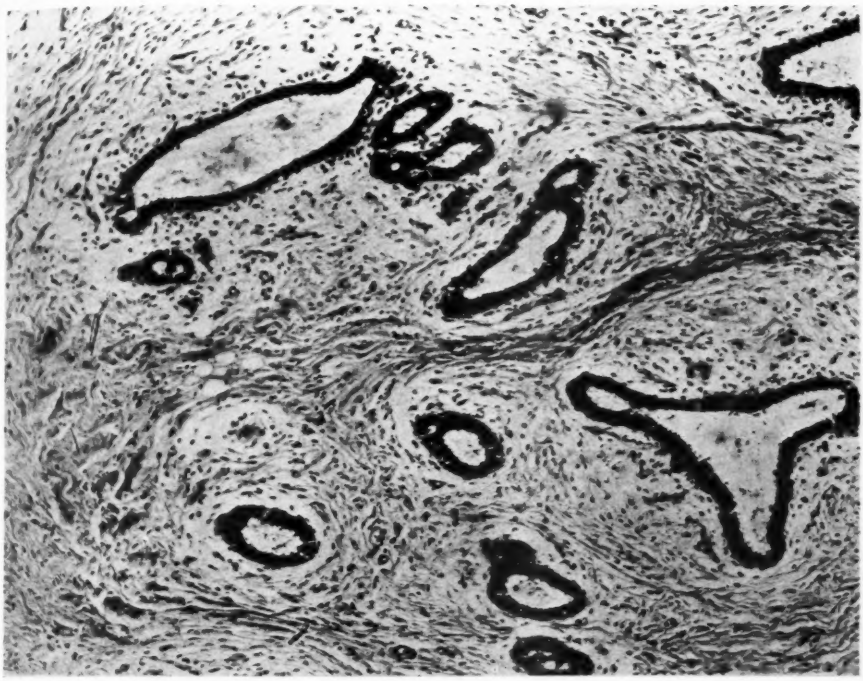
Laipply and Shipley Extragenital Choriocarcinoma in the Male

PLATE 156

FIG. 5. Ducts of breast are increased in number and lined with stratified columnar epithelium. Loose cellular periductal connective tissue is infiltrated with lymphocytes. Hematoxylin and eosin stain. × 94.

FIG. 6. Atrophy and hyalinization of seminiferous tubules and hyperplasia of interstitial cells of testis are evident. Hematoxylin and eosin stain. × 140.

FIG. 7. Marked hyperplasia and pleomorphism of interstitial cells of testis. Hematoxylin and eosin stain. × 140.



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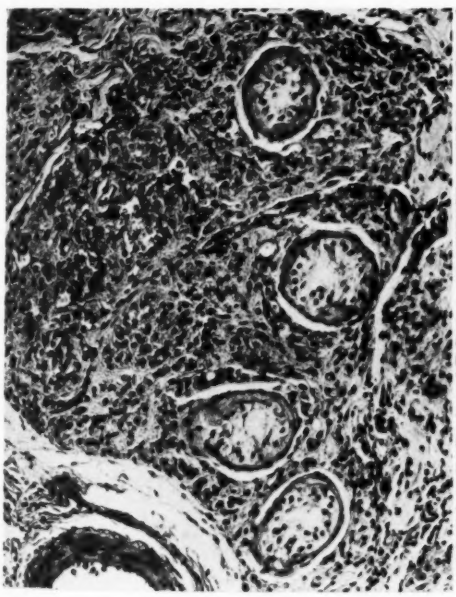

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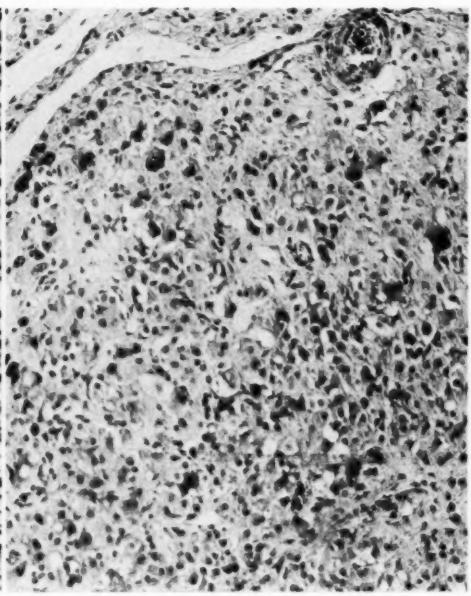

7

Laipply and Shipley

Extragenital Choriocarcinoma in the Male

UNIV. OF MICH.

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

VI. Pathologic Effects of Traumatic Shock on Motor and Sensory Nerve Endings in Skeletal Muscle of Unanesthetized Rats in the Noble-Collip Drum *

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The morphologic effects of traumatic shock upon the motor and sensory nerve endings in skeletal muscle are unknown. Morphologic evidence has been presented in previous studies [1] which supports the statements that the pleomorphism of the hypolemmal axons of the motor end-plates is due to ameboid motion and that the changes in amount of the granules of Kühne are evidence of different phases of periodic activity of the secretory mechanism of the motor end-plates. Evidence has likewise been presented that there is a metabolic destruction of the motor end-plates by lactic and other acids.[2] There is, furthermore, an acute anatomic breakdown of motor end-plates in hemorrhagic shock.[3] The lesions of the motor end-plates in poliomyelitis,[1] in the experimental injection locally of lactic acid,[2] and in hemorrhagic shock [3] are quite similar. When shock is produced by hemorrhage,[4] trauma,[5] or gravity [6] there is an accumulation of lactic acid and keto-acids in the blood. Gesell and his colleagues [7] have presented important evidence that an accumulation of lactic acid retards the destruction of acetylcholine at the synapses. This local increase of metabolic acids at the neuromuscular apparatus may play a rôle in the alterations of the junction between nerve and skeletal muscle. Shock produced by direct hammer trauma of the living, skin-intact muscle [8] is similar in some respects to the traumatic effect of the Noble-Collip drum on the neuromuscular apparatus. The morphologic effects of shock from any cause upon the neuromuscular apparatus of skeletal muscle have remained an unexplored field. A closer study of the pathology of this region may help to explain the mystery of the onset of shock. Cannon [9] (page xi) in his excellent monograph on shock stated:

"....the mystery of the onset of shock has not been definitely cleared away despite a considerable increase in our knowledge of it, and that there still remains

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much work to be done before we shall have elucidated all the factors which play a rôle in its establishment."

and, on page 111:

"An explanation for the development of a low blood pressure, apart from hemorrhage, has not been offered. It is at this point that most diverse opinions arise. Many theories have been offered, often with experimental support, to account for the development of low blood pressure."

Moon [10] in his comprehensive, thought-provoking review of the subject of shock pointed out the significant fact that the pathology of shock was an ignored subject until his own experimental studies. He made the following significant statements (page ix):

"A survey of recorded studies on shock reveals the remarkable fact that in no instance had it been investigated from the standpoint of pathology. Probably this was because it was interpreted entirely as a functional disorder having no morphologic basis. The belief that post-mortem examinations reveal no significant changes following death from shock, had been accepted apparently without question. The problem might have been clarified earlier if the methods of pathology had been combined with those of physiology in the search for pertinent evidence. In this as in other conditions of disease, valid interpretations find corroboration in the accompanying morphologic changes."

In the present state of the lack of knowledge of the pathologic effects of traumatic shock on the secretory mechanism of the motor end-plates, an adequate number of clear-cut photomicrographs is necessary. Concrete evidences of the morphologic changes of the motor end-plates resulting from trauma reveal more specifically than words that there is an ephemeral and tenuous secretory liquid transmitted at the junction between motor nerves and skeletal muscle. By teasing large amounts of muscle, various phases in the pathologic alterations of this secretion are found. By teasing muscle after gold impregnation, the anatomic relationship and the polarity of the secretory mechanism of the neuromuscular apparatus are preserved. The relationships of the epilemmal axon, hypolemmal axon, ramifications of the hypolemmal axon, the discharged granules of Kühne, and the related cross striations of the muscle fiber are revealed in one observation. This anatomic arrangement is obscured by the sectioning method even though the cytologic details of the nuclei and neurofibrils are better revealed by sectioning than by the gold-and-teasing method. Both methods, therefore, should be employed in the study of the neuromuscular apparatus.

A pertinent statement in reference to the gold method, written by Kühne [11] (page 3) in 1887, may be translated as follows:

So much has been written about the use of gold in the staining of muscle and nerve fibers that one has good cause to be brief about it, in particular, since those who have used the method themselves know only too well how little can be learned from numerous descriptions of the procedure and that only individual trial and long experience with the method can lead to sure results. Even the beginner can experiment with making motor nerve endings visible by the gold method, utilizing the action of a reducing medium with which is linked the name of Cohnheim, who

first in histology had success with the gold method; how the gold picture comes out and what structural secrets of nerve endings it hides is still unsatisfactorily known, regardless of the abundance of experimentation with it.

The terminations of nerves at synapses and in muscles and glands have been difficult to study in the past because of technical limitations and more especially because of the unappreciated inconstancy of the structural arrangement related to the different states of the secretory activity stopped by death. Bremer [12] made the following statements:

"Like all nerve endings they [motor endings] are difficult to study because of the unreliability of the staining methods necessary to bring out the fine nerve fibers as distinct from the tissues among which they run. Impregnation methods [silver and gold] and supravital staining with methylene blue are most commonly used, but both require great care in interpretation of results."

The gold-and-teasing method may be easily mastered with persistency and experience. When the objective findings are presented, anyone may make the interpretation.

The purpose of this paper, therefore, is the presentation of direct and conclusive experimental morphologic evidence, in the form of easily verified and clear-cut, untouched photomicrographs, that supports the following theses: (1) that the experimental pleomorphism of the hypolemmal axons of the motor end-plates is the result of normal and abnormal functional ameboid motion; (2) that the experimental variation in the quantity of the granules of the sole plate of Kühne is the structural expression of the differential phases in the secretion of a chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates; (3) that the gold-staining axonic inclusion masses found within and between skeletal muscle fibers is possibly the morphologic expression of the delayed dissolution of acetylcholine; (4) that traumatic shock produced in unanesthetized rats in the Noble-Collip [13] drum results in the abnormal secretion of axonic substance into the muscle, which secretion, in some instances, leads to a loss of motor innervation at the myoneural junction by exhaustion due to hypersecretion of the nervous liquid; and (5) that the pathologic changes of the muscle fibers in traumatic shock are evidence of the profound underlying changes in the biochemical status of the muscle.

Materials and Methods

The gold chloride method previously described,[14] followed by gentle teasing of the motor nerves and muscle fibers, is superior to any other method for our purposes. It reveals the subtle and evanescent neuronal secretion of the motor end-plates in the skeletal muscle fiber as well as the anatomic relationship and polarity of the motor end-plate, epilemmal axon, granules of Kühne, and the cross striations of the muscle fiber. The motor end-plates in the gastrocnemius muscle of the white

rat (*Mus norvegicus*) were stained also by the Bielschowsky method of silver impregnation as modified by Boeke; [15] by the *intra vitam* methylene blue method of Ehrlich as modified by Huber,[16] and by Huber and DeWitt; [17] and by the Ranvier gold chloride method as modified by Wilkinson [18] and by one of us.[14] The spinal cord and brain from traumatized rats were serially sectioned and stained for Nissl substance.

The Bielschowsky silver technic reveals the fine neurofibrillar, reticular nature of the fronds of the axon. The sarcoplasm of the sole plate is clear and unstained by methylene blue. The gold chloride method, however, demonstrates the sole plate (when it is present) to be composed of fine granules. There is a narrow, clear area between the fronds or branches of the terminals and the granular sole plate in some places. In certain expanded plates there is direct continuity between the serrated projections of the terminations of the axon and the dark muscle striae. The ovoid nuclei of the sole plate remain unstained by the gold method and form clear spaces surrounded by dark granules. The periterminal network of Boeke is best seen in expanded nerve plates after staining by the silver method. It forms a foamlike reticulum between that of the nerve terminals in the plate and the periterminal network in the sarcoplasm of the sole plate. In the expanded nerve plates, which appear to be chemical secretory apparatus, there is an apparent finer structural continuity between the terminal nerve plate and the surrounding granular sole plate when stained by the silver method. In the retracted plates this apparent continuity is lost. The line of demarcation between nerve terminals and sarcoplasm is not a constant one. It depends upon the state of functional activity in which life is stopped and the tissue fixed. Although the structural integrity of both muscle and nerve is preserved at the myoneural junction, it appears that some chemical secretory granules pass from the nerve end to the muscle in some rhythmic manner. Are these granules acetylcholine? Evidence is accumulating which supports an affirmative answer.

It may be that the initial use of lemon juice, with its citric acid content, to increase the permeability of the fresh muscle and nerve tissue for gold chloride likewise prevents or retards the dissolution of acetylcholine. For this reason the anatomic demonstration of hyperchromatic gold-impregnated masses in the muscle fiber may be specific for acetylcholine or some related substance discharged from nerve into muscle. Gesell and co-workers [7] have found that increase of acidity by lactic acid retards the destruction of acetylcholine physiologically deposited at the synapses. The other histologic methods with silver, methylene blue, osmic acid, and hematoxylin and eosin, followed by sectioning, do not conserve the normal and abnormal morphology of the neuromuscular apparatus as does the modified gold technic.

One hundred and ten white rats were used in this study and over 2500 slides of teased muscle were obtained from a survey of over 20,000 slides. Experimental traumatic shock without hemorrhage was produced in unanesthetized white rats, average weight 200 gm., in the Noble-Collip [13] drum (Figs. 1 and 2). The underlying principle of the Noble-Collip drum is to traumatize the rat by placing it in a revolving circular drum in which are projections or bumps. These carry the animal up the side during a turn and then drop it, to be picked up by the following projection. The distance the animal falls is such that obvious hemorrhage or fracture of the bone is not produced. The drum has an inside diameter of 18 inches and a depth of 9 inches. The projections are of a blunt triangular shape on cross section with a height of 2 inches and a base of 3 inches. The outside of the drum is closed by a circular, hinged lid in which there is a cellophane window for observation of the animals during the revolution of the drum. The animals used were maintained under standard conditions. The rats which survived were killed at various intervals after traumatization. Post-mortem examinations were made on all animals and our findings agree essentially with those reported by Noble and Collip. They found the most satisfactory drum speed to be 40 r.p.m. In their male rats there was a 100 per cent mortality within an average time of 50 minutes after 700 revolutions. In female rats there was a 75 per cent mortality in the average time of 81 minutes after 700 revolutions. They likewise found that when the rats were subjected to 800 revolutions there was a 100 per cent mortality in the average time of 47 minutes in the male and a 100 per cent mortality in the average time of 39 minutes in the female. The time of death after shock increased as the percentage of mortality and number of revolutions became reduced.

We agree with Noble and Collip [13] that the death or survival of the animal is the most accurate indicator for experimental shock. Blood pressure changes are influenced by anesthesia and hemorrhage. Blood volume measurements are uncertain through the loss of the dye into the tissues. Hemorrhage influences both the red cell counts and hemoglobin determinations. Many of the chemical changes may be interpreted as secondary. Rectal temperatures below 95°F. are frequently found as well as severe hemoconcentration. Rats removed from the drum after 700 or 800 revolutions were either listless or in ante-mortem rigor and appeared ill, but were not unconscious. Respiratory rate was usually increased, slight hemorrhage might be found around the mouth and nose, and in some the incisor teeth were broken. In animals that die soon after removal from the drum the muscles may be either flaccid or in a condition of ante-mortem rigor. Some muscles manifested pronounced fasciculation. The respirations became progressively slowed.

There was considerable variation in the time of appearance of the signs and symptoms of shock, a fact which confirms previous findings of the variable resistance of rats to trauma.

The changes seen in the various organs vary in intensity depending upon the degree of trauma and the time of death after trauma. The muscles in general appear redder and more vascular than normal. There is extreme vascular dilatation, engorgement, and congestion of the mesentery and bowel. Petechial hemorrhages occur quite frequently in the stomach and small and large intestine. The kidneys are usually congested and some specimens of urine contain blood. The spleen is frequently enlarged and congested. The blood is dark-colored and thick in the liver, heart, and lungs. These visceral changes are not very striking in animals that die within 5 to 10 minutes after trauma. Histologically the viscera present changes comparable to those reported by Noble and Collip [13] and by other investigators.

The pathologic effects of hemorrhagic shock [3] on the neuromuscular apparatus in the gastrocnemius muscle of the white rat have been reported. The effects on the motor end-plates of traumatic shock produced by the Noble-Collip drum have not been reported heretofore. The 110 rats that constitute the basis of this report were divided into four series of 25 rats each and one series of 10 used as controls. Series I was composed of those rats that were killed by decapitation immediately after 400 revolutions in the Noble-Collip drum rotating at the rate of 40 r.p.m.; series II, the rats that were killed within 20 minutes, after 800 revolutions; series III, the rats that were killed 30 minutes after completion of 800 revolutions; and series IV, the rats that were killed 40 to 60 minutes after completion of 800 revolutions.

Moon [10] stated that hemorrhage, infection, and anesthesia were complicating variables involved in the different experimental methods previously used to produce direct traumatic shock. Noble and Collip [13] have devised a method in which trauma is applied in a graded, controlled manner, following which a typical picture of shock developed, uncomplicated by hemorrhage, infection, or anesthesia.

Results: Experimental Findings

1. The Pleomorphism of the Normal Motor End-Plate

It was a difficult problem to establish an absolute standard for the structure of the normal motor end-plates. These varied to the point of complete dissolution, in correlation with the elapsed time after death and the degree of activity prior to death. During post-mortem rigidity a rapid disappearance of the motor end-plates occurred in skele-

tal muscle, associated with a considerable local increase in hydrogen ion concentration. This increase was due mainly to a local accumulation of lactic acid, which usually reaches a concentration of from 0.05 to 0.3 per cent in rigor mortis according to the findings of Fletcher and Hopkins.[19] The local increase of acidity by the injection of lactic acid produced rapid dissolution of the motor end-plates.

These findings indicated that the end-plates must be studied as early after death as possible and preferably from muscles excised from animals prior to the cessation of cardiac and respiratory activity. Various anesthetic agents such as ether, chloroform, and pentobarbital sodium modified the structure of the end-plate. It was necessary, therefore, to know in detail the means of death, such as anesthetic agent, bleeding, decapitation, and fatal trauma, in establishing a relative morphologic norm for the motor end-plate. Under light anesthesia (4 mg. pentobarbital sodium per kg. injected intraperitoneally), both gastrocnemii muscles in 10 rats (average weight 200 gm.) were excised 15 minutes after the injection of the anesthetic agent. The muscles were immediately processed by the gold technic.

The length of these relatively normal motor end-plates measured in the long axis of the muscle fibers in the gastrocnemius muscle varied from 20 to 60 μ; the breadth was from 10 to 40 μ and the thickness from 5 to 15 μ. The mean for the length of 1000 relatively normal motor end-plates was 30 μ, and the mean for the width, 22 μ. The mean of the diameter of 1000 muscle fibers was 52 μ. In size and shape these normal motor end-plates, therefore, were highly variable (Fig. 4). There was a quantitative change of the granules of Kühne in the sole plate. They were increased in amount in the relatively retracted end-plates related to muscle relaxation and had an increased staining capacity for gold. In the normally expanded end-plates, associated with muscle contraction, the granules were decreased in amount and more dispersed than in the retracted end-plates. The variations in the size, shape, and number of the branches of the hypolemmal axons of the motor end-plates have been presented previously as evidence of an underlying ameboid motion. The differences in the amount and capacity to take the gold impregnation of the granules of Kühne were concrete evidence of a subtle secretion process that occurred periodically at the neuromuscular apparatus.

The normal retracted axons of the end-plate under high magnification had a rim of Kühne's granules and an intervening light space in some places (Fig. 44). The normal expanded axons of the end-plate, under high magnification, had a diminution or complete absence of the surrounding rim of Kühne's granules (Fig. 46). Although these rela-

tively normal retracted and expanded motor end-plates were obtained from trees of innervation during the early stages after they were subjected to trauma, they were very similar to those obtained from the normal motor nerve tree (Fig. 4). Normal and abnormal motor end-plates were intermingled on the motor nerve trees during the early stages of muscle trauma. There was a progressive pathologic change leading to complete denudation of epilemmal axons of end-plates in the later stages of muscle trauma. This depended upon the intensity and duration of the traumatic stimulus and variation of resistance to trauma of the individual living organism.

2. The Experimental Production of Axonorrhea of the Motor End-Plates in Traumatic Shock

The best time to detect axonorrhea, or the enormous outpouring of axonic material into the muscle fiber from motor nerve endings increased in permeability, was between 5 and 15 minutes after the animals had been subjected for 20 minutes to 800 revolutions in the Noble-Collip drum. Large quantities of muscle must be teased in order to detect this phase in the hypersecretion from the motor end-plate. This axonic material, discharged from the motor end-plates, had an intense affinity for gold, taking a more intense impregnation than the cross striations of the muscle fiber. It was found both under the sarcolemma within the muscle fiber, and outside of the sarcolemma between the muscle fibers. This secreted axonic material varied in length from small granules of 0.5 μ to elongated masses of 1000 μ, and in width from 0.5 to 60 μ (Figs. 3, 5, 7, 13, 16, 24 to 27, 39 to 41, 43, and 64 to 68, Kg.). The internal structure of this axonic material was either homogeneous or cross-striated. In some locations the cross striations agreed with the periodicity of those of the muscle fiber. In other locations there was no agreement between these cross striations of the extra-axonic material and those of the muscle fiber.

In certain trees of motor innervation various steps in the descent and discharge of axonic material were followed through the epilemmal axons, motor end-plate, and into the muscle fiber (Figs. 9, 11 to 14, 18 to 29, and 36 to 41). Some of the end-plates had a single island of gold-chromatic material in the center which gave the appearance of the hub of a wheel (Figs. 34 and 45). Other plates were expanded and open in the center (Fig. 35). Some were relatively normal, with retracted hypolemmal axons surrounded by a dense rim of Kühne's granules (Fig. 44), or expanded, with a decrease in the amount of Kühne's granules (Fig. 46). Various steps in the descent of the hyperchromatic gold material through the epilemmal axons to the end-plates were like-

wise clearly evident in various nerves of the same tree of innervation (Figs. 46 to 48). In some places the epilemmal axon was beaded and very little axonic material was found in the hypolemmal axons of the motor end-plate. On the other hand, in neighboring end-plates of the same tree of innervation, large amounts of axonic material were found. This quantitative increase of the gold-chromatic material in the end-plates was detected by the intense impregnation. In other end-plates the beginning of the discharge of the gold-chromatic material was detected extrinsic to the hypolemmal axons of the end-plate. The gold-chromatic material, therefore, was either intra-axonic or extra-axonic. That which was extrinsic to the axons of the end-plate was either in small or large fusiform masses (Figs. 7, 14, 27, 40, 41, and 62 to 68), globules, or droplets (Figs. 5, 6, 9 to 12, 20, 22, 23, 29 to 33, and 36 to 38). The arrangement of the discharged axonic material was unipolar (Figs. 22, 25, and 37), bipolar (Figs. 23, 26, and 36), or multipolar (Figs. 13, 21, 24, and 27).

Within 5 to 15 minutes after the rat had revolved 800 times in the drum some of the end-plates of the motor trees had an increase of the gold-chromatic material as detected by augmented impregnation (Figs. 5 to 11, 16 to 20, 42, and 43). There was a progressive decrease to the point of complete disappearance of the gold-chromatic material in the hypolemmal axons of many end-plates (Figs. 8, and 49 to 57). In some motor trees the gold-chromatic material had disappeared from both the epilemmal and the hypolemmal axons of the motor end-plates (Fig. 58). The process of hypersecretion, therefore, of the axonic material into the muscle produced complete disappearance of the motor end-plates (Figs. 8 and 59), and a gradual depletion of the axonic material in the epilemmal axons in a centripetal direction from the end-plate. In some muscle fibers the liquefaction leading to disappearance of the motor end-plates was indicated by a corresponding degeneration and liquefaction locally of the cross striations of the related muscle fiber (Fig. 8). The epilemmal axons first became beaded, then fragmented, and finally there was complete disappearance of the gold-chromatic material.

The rat in the terminal stage of traumatic shock, decapitated 1 hour after 800 revolutions during 20 minutes in the Noble-Collip drum, had 3,124 end-plates denuded from 3,500 epilemmal axons that were counted in the gastrocnemius muscle (Fig. 8). This denervation of muscle at the myoneural junction was comparable to that produced by experimental poliomyelitis in the monkey, poliomyelitis in human muscles, and by the injection of lactic acid locally in the zone of motor innervation of skeletal muscle in the rat. The number of epilemmal

axons denuded of end-plates or possessing pathologic end-plates varied in different fibers within the same muscle as well as in different muscles in the same rat. The variable time factor of denudation of the nerve fibers of their end-plates was, therefore, important: the longer the time after muscle trauma the greater the quantity of end-plates that underwent complete disappearance. There was individual variation of the rats in manifesting this deviation from normal.

The rats of series I that were killed immediately after 400 revolutions in the drum at the rate of 40 r.p.m. had 89 end-plates in a state of extensive axonorrhea in a differential count of 3,156 end-plates in gastrocnemius muscle taken at random. There were individual variations in the animals. In two animals there was no extensive axonorrhea (abnormal discharge from the permeable end-plates) and in one animal there were 10 plates of a tree with 60 endings manifesting extensive discharge of the gold-chromatic material in one of the gastrocnemius muscles. Many end-plates had an increased intensity of gold impregnation (hyperchrysophilia).

The rats of series II that were killed within 20 minutes after 800 revolutions in the drum rotating at the rate of 40 r.p.m. had 267 end-plates in a state of extensive axonorrhea in a differential count of 3,097 end-plates counted in gastrocnemius muscle taken at random. Again there was individual variation in the animals. In one muscle there were 5 motor end-plates manifesting extensive discharge, and in another there were 20 of a tree with 45 endings in a state of extensive axonorrhea. Many end-plates had a decreased intensity of gold impregnation (hypochrysophilia).

The rats of series III and IV that were killed 30 minutes and 40 to 60 minutes, respectively, after 800 revolutions in the drum rotating at the rate of 40 r.p.m. had the majority of the end-plates in various degrees of liquefaction. Extra-axonic gold-impregnated masses (*neurosomes*), discharged from the end-plates into the muscle fibers, were rarely found. The usual appearance of the motor innervation was one in which the epilemmal axons of the motor trees were almost completely denuded of the motor end-plates (Figs. 8 and 59). There were 3,124 end-plates denuded from 3,500 epilemmal axons counted in muscle taken at random and excised 1 hour after rotation in the drum.

3. *Effect of Trauma on Muscle Fibers and Blood Vessels*

Dependent upon the time of observation after muscle trauma and individual variations, some rats were flaccid and others rigid while respiratory and cardiac activities were still going on. Certain localized groups of muscles manifested fasciculation and others periodic con-

vulsive movements. The functional condition of the muscles, therefore, was pathologic and highly irregular in traumatic shock. In the final stages of irreversible shock stimulation of the sciatic nerve failed in many instances to cause a response in the gastrocnemius muscle and in certain other muscles. From the motor innervation in the late stages of shock there was almost complete disappearance of the end-plates (Figs. 8 and 59). Certain other muscles in the end stage of shock were in a greatly reduced state of irritability or had lost completely the capacity to respond to direct electric or mechanical stimulation. Many muscle fibers whose irritability was reduced or lost had so-called Zenker's waxy and granular degeneration (Figs. 8, and 59 to 62). There was a correlation between flaccid muscular paralysis and peripheral nervous exhaustion of the structure at the myoneural junction. Some of the muscle fibers had a series of fusiform structures that were intensely impregnated with gold (Figs. 15 and 61). There were irregularities in arrangement and loss of the cross striations, increased visibility of pyknotic nuclei, and perivascular infiltration of leukocytes (Figs. 15, and 59 to 61). There was a disappearance of the differential fiber types in both a localized and generalized manner. The sarcoplasmic substance in some muscle fibers was increased and in others decreased. Some of the muscle fibers were swollen. In other locations the muscles grossly not only appeared more red than normally but upon microscopic observation the intramuscular capillaries and venules were dilated and contained agglutinated red blood cells accompanied by perivascular edema. In other places these vessels were collapsed and devoid of cellular constituents.

4. Effect of Trauma on the Proprioceptive Sensory Muscle-Spindles

There are two to ten intrafusal muscle fibers in each sensory spindle. Each intrafusal muscle fiber maintains connection with two types of nerve fibers: (1) with the large sensory medullated nerve or nerves which arborize in spirals, dendritic branchings, and rings in the encapsulated enlargement, and which conduct proprioceptive afferent impulses upon stretching and increasing the tension of the muscle (Figs. 69 to 71, M.S.); and (2) with the motor medullated nerves which conduct efferent centrifugal impulses by virtue of which the intrafusal muscle fibers of the sensory spindle are capable of normal and abnormal contraction like the other ordinary extrafusal muscle fibers.

In some of the muscle spindles the terminal nerve organ was fragmented by trauma and in other locations the nerve terminals were intact. The most evident pathologic finding was periodic dilatation or beading (Fig. 69, D) of the axons which conduct the sensory impulses

away from the spindle. This moniliform pattern of the sensory axons was apparently morphologic evidence of hyperactivity of the sensory muscle spindle due to trauma. The tendon spindle (Fig. 72, T.S.) gave slight evidence of fragmentation of the end-organ which terminated in globular enlargements of the axons and slight beading of the afferent conduction axon which indicated overactivity.

5. *The Effect of Trauma on the Motor Cell Bodies in the Ventral Horn of the Spinal Cord*

Under the conditions of the experiment of a short time in the Noble-Collip drum and subsequent death, the anatomic changes of the ventral horn cells in the cord were not so advanced as those demonstrated in the motor end-plates in the muscle of the same animal. During the early stage of trauma many of the cell bodies manifested chromatolysis of the Nissl substance in the cytoplasm. In the late stage of traumatic shock there was slight shrinkage of many motor cell bodies and beginning vacuolization of the cytoplasm. The nucleolus was hyperchromatic but the rest of the chromatic material and the cytoplasm had disappeared from many motor cells. No cell was found in a stage of complete cytolysis, probably because the elapsed time after trauma was too short. Many of the cells were hyperchromatic in animals of series I killed immediately after 400 revolutions in the drum rotating at the rate of 40 r.p.m. On the other hand, the majority of the cells were hypochromatic in the cords of animals killed 40 minutes to 1 hour after 800 revolutions.

Discussion

The pathologic effects of traumatic shock on the neuromuscular apparatus in voluntary muscle amplify the normal neuronal secretion to the point of complete depletion of the terminations of the peripheral nervous motor mechanism and the production, in places, of granular degeneration of the muscle substance. The process of elaboration and discharge of the abnormal neuronal secretion is recognized by changes in the form and contents of the motor end-plates. Under normal conditions this granular secretion is ephemeral and tenuous; the quantitative changes of the granules of Kühne are evidence of it. During the early stages of the abnormal conditions there appear to be both an increase in the amount of the neuronal secretion and a delay in its dispersal and disappearance. During muscle traumatization, discharge of the nervous secretion appears to proceed more rapidly than its elaboration. The process involves a certain polarity of the motor end-plates. The basal pole is directed toward the sarcolemma and receives, through the epilemmal axon, the supply of nutritive material which is

elaborated in the ventral horn cells and along the course of medullated nerves. The secretion is passed out from the opposite or free pole of the ramifications of the hypolemmal axons into the myoplasm. The abnormal extra-axonic secretion accumulates as discrete granules or as homogeneous or striated masses of these granules, which form streamers of various sizes both within and without the muscle fiber.

This polarity of the secretory mechanism of the neuromuscular apparatus is best studied, therefore, in its entirety, in teased preparations impregnated with gold, and not in the ordinary sectioned specimens. When such gold-impregnated, teased specimens from traumatized muscles are examined microscopically, the nuclei of the granular sole plate of Kühne are seen to be either vesicular with distinct chromatin granules, or pyknotic. The morphologic changes in the motor end-plates may simulate those of a holocrine, apocrine, or merocrine gland. Under certain experimental conditions of overstimulation by physical or chemical means the hypolemmal axons of some motor end-plates disintegrate en masse into the secretory granules, comparable to the morphologic changes of a sebaceous gland cell, a holocrine type of gland. We have designated as "axonorrhea" the condition of abnormal outpouring of the axonic material in elongated streamers from the motor end-plates into the myoplasm. The morphologic changes in the nerve endings and muscle fiber are substantial evidence of underlying biochemical alterations in the neuromuscular apparatus. There is morphologic evidence of the chemical theories of the transmission of the nerve impulse proposed by DuBois-Reymond,[20] Langley,[21] Loewi,[22] Dale and Feldberg,[23] Cannon,[24] Gesell, Brassfield, Hansen, and Mason,[25] and others. These pathologic findings in the nerve endings and muscle fibers in voluntary muscle may be involved in the onset of traumatic shock and, furthermore, may be the structural expressions of the initial elaboration of toxic substances in voluntary muscle.

It is interesting to note that during and subsequent to the Civil War in the United States there were theories concerning shock which involved the idea that there was an exhaustion of some subtle nervous fluid. This conception had been discarded until recently because of the lack of physiologic and especially morphologic evidence. In the monograph on shock by Moon [10] (page 95), the following appears:

"The earlier hypotheses were entirely conjectural. Foreign substances in wounds, coagulation and other changes in the blood were proposed as causes. The writings abound in such hypothetical concepts as 'draining of vital fluid,' 'loss of animal and organic powers,' 'destruction of the great nervous power,' 'complete depression of all vital functions,' 'commotio cerebri,' and the like. A detailed review of these theories is not profitable. One instance may be cited as reflecting the character of medical thought within this period. Samuel D. Gross (1872) wrote: 'Shock is a depression of the vital powers, induced suddenly by external injury,

and essentially dependent on loss of innervation. It bears the same relation to the nervous system as syncope to the vascular. In the one case the result is caused by a diminution of the nervous fluid, in the other by a diminution of the blood; in both the consequence is more or less prostration. ... The blood has long been known by physiologists as the vital fluid so necessary to the well being of the system. But it is certainly not the only fluid entitled to this distinction; the nervous fluid is both more subtle and more important as a life preserver. When blood flows away in a mighty and overwhelming torrent ... life is destroyed by the excessive sanguinous drainage. But in shock the same effect may happen, and yet the body be literally surcharged with blood, not a single drop, perhaps, having been spilled in the accident causing the fatal result. Thus of the two fluids the nervous is the more important, because the more essential to life; and its disturbance is therefore a more frequent cause of death.' "

Gross based his hypothetical concepts of "loss of innervation" and "diminution of nervous fluid" upon clinical observation. He was nearer the truth than most morphologists have realized up to the present time. The same applies to the prophetic and intuitive statement, "is it that conduction checked somewhere causes at that spot destruction from dangerous accumulations of nerve force?", by that acute observer and clinician, S. Weir Mitchell,[26] in 1872 (pages 142–144), more fully expressed as follows:

"Shock, then, is reflex disturbance, or, in some cases, paralysis of centres. Why in one case the cerebrum should suffer, in another the heart, and in a third the motor centres of the leg or arm, is as yet inscrutable. A ball crushes a nerve, and the tremendous shock instantly propagated to the spine falls ruinously upon some one of the numerous ganglia through which it travels. Is this because it finds a weak point, or is it that conduction checked somewhere causes at that spot destruction from dangerous accumulations of nerve force? . . . .

"These, also, I prefer to explain by presuming that the shock had suddenly exhausted some ganglionic centre, and thus palsied its related muscles. This view was set forth by us in March, 1864, and more elaborately, though not more distinctly, by Jaccoud, in December of the same year. We then remarked that 'either the shock of a wound causes paralysis of vaso-motor nerves and sequent congestion, with secondary alterations, or that it destroys directly the vital power of a centre. Now, there is no reason why, if shock be competent to destroy vitality in vaso-motor centres or nerves, it should be incompetent so to affect the centres of motion or sensation.' Indeed, it appears incomprehensible that any vasal spasm and consequent relaxation could be competent to instantly and permanently paralyze a whole limb, while sudden deaths from shock seem also explicable in no other way than by absolute exhaustion of nerve force in some vital centre."

If the term "vital centre" be replaced by the one "motor end-plates," Mitchell's speculation has anatomic evidence to support its validity. Mitchell,[27] in 1866 (page 355), came to the conclusion that muscle paralysis due to shock was based on nervous exhaustion:

"If I were now to sum up the probabilities in the way of causation of palsies peripherally induced, I should be disposed to refer some cases to exhaustion from too constant or excessive exercise of normal functions, and others to irritation from disease or injury, and to consequent exhaustion of the centres; while, as regards the intervention of vascular agency, I should reject the idea of prolonged vasal spasm, and consider it possible that in some instances over-excitation might

result in dilatation of the vessels, in which case some material lesion would surely follow, if the condition in question were of long continuance."

There is abundant morphologic evidence that traumatized muscle has demonstrable pathologic changes in both the motor end-plates and muscle fibers. These alterations may be the structural expression of profound biochemical changes in the neuromuscular apparatus that may be involved, likewise, in the production of abnormal amounts of certain toxic metabolites. Green and Bielschowsky [28] claimed that adenosine triphosphate may be this toxic substance. In the following statements (page 197) Noble and Collip [13] supported the hypothesis that a toxic substance is produced in the tissues due to trauma:

"The findings which have been obtained up to the present are most readily interpreted by the hypothesis which has been advanced by many that a toxic substance is produced in the tissues due to trauma. If this is the case it would explain why some animals may die immediately following trauma and why, once this type of trauma ceases, the production of a toxic substance stops. In the animals where death follows almost at once the typical shock picture may be only partially developed and haemoconcentration present only to a slight degree. These features must therefore be secondary to the liberation of the toxic substance. It would appear that if enough of the toxic substance enters the blood stream it produces death rapidly, possibly by affecting the heart or brain. In an animal receiving trauma for a short period the peripheral circulation is probably relatively good and therefore the toxic substance may be rapidly removed from the damaged tissue to the blood stream. On the other hand, in an animal subjected to trauma over a prolonged period the circulation may be poor and the toxic product released slowly causing death at a later interval. Somewhat similar conditions may influence the concentration of the blood. In animals dying rapidly little haemoconcentration may be found. However, in the presence of traumatized tissues, over a longer period, marked loss of plasma from the blood may occur. It would appear that if the toxic substance produced remains in the tissues, as when the peripheral circulation is poor, the loss of fluid locally is marked. In such cases the death of the animal is related to a direct toxic effect plus the result of haemoconcentration. Further, when haemoconcentration is advanced the oxygen supply to the peripheral tissues is deficient. Such anoxaemia may lead to a further local increase in the production of the toxic substance. Such an argument would correlate the type of shock caused by methods employing temporary occlusion of the blood supply to some part of the body with that following direct trauma, since the end result would be the liberation of the same toxic product. The above theory for the aetiology of the development of shock, which has been evolved from the experiments described and others to be published, is similar in some ways to that of Moon. However, in the theory outlined the peripheral tissues are suggested as the site where some fundamental chemical alteration occurs following trauma or ischaemia, and this results in the production of a toxic substance. This substance may act locally, resulting in the attraction of plasma with haemoconcentration (which in turn causes secondary general ischaemia and additional liberation of the toxic material), or systemically, possibly affecting the heart and brain, and even producing rapid death. Whether the substance acts locally or enters the systemic system may in itself be determined or influenced by the state of the cardiovascular system and peripheral circulation. In such a plan the capillaries would have a passive rôle and be affected secondarily rather than be the primary site of the damage responsible for the development of the shock picture, as outlined by Moon.

"If this explanation is correct the types of shock, as seen both experimentally

and clinically, might be expected to show marked variations. At the one extreme would be cases where death followed trauma very rapidly, and at the other those which ran a protracted course and in which increasing haemoconcentration was a prominent finding. Therapeutic procedures which tend to alleviate increased blood concentration might be expected, therefore, to be beneficial in the latter type of case only."

Possibly when the pathologic mechanism of shock due to trauma, hyperthermia, hemorrhage, or infection is completely known the voluntary neuromuscular factor may be found closely related to the production of toxins (Moon,[10] Dale,[29] Cannon,[30] Bayliss [31]) and to local fluid loss from capillaries (Blalock,[32] Harkins,[33] Freeman, Freedman, and Miller [34]).

The concept of an axonal secretion of the motor end-plates discharged into the muscle fibers requires a reconsideration of the physical nature of the conduction medium of motor nerves in skeletal muscle. The physical nature of the motor axis cylinder and the means by which the nerve impulse is conducted and discharged in muscle are unknown. After Remak [35] discovered neurofibrils in the living nerve fibers of the crayfish, Schultze [36] presented the hypothesis that the neurofibrils were the specific wire-like conductors of the nerve impulse. What went up or down the neurofibrils to constitute a nerve impulse was unknown.

The prevalent opinion expressed in most textbooks of histology is that the neurofibrils are the specific core electroconductors of the neural impulse. Neurofibrils were found by de Rényi [37] in living nerve fibers of invertebrates. Parker [38] stated that the neurofibrils control the metabolic trophic activities of the neuron, whereas Verworn,[39] von Lenhossék,[40] Koltzoff,[41] Goldschmidt,[42] and von Szüts [43] held that the neurofibrils in the axon are supporting structures for the semifluid neuroplasm.

The theory that the conduction medium of the nerve impulse is a liquid is supported by the observations of Jenkins and Carlson,[44] Carlson,[45] Meek and Leaper,[46] Lapicque and Legendre,[47] Erlanger,[48] Erlanger and Gasser,[49] Gasser and Erlanger,[50] Young,[51] Gerard,[52] and Weiss.[53]

In our own experimental studies [54] evidence was produced that supported the following statements (page 428):

"The physical state of the axis-cylinders in the central and peripheral nervous systems is that of a colloidal liquid. The experimental production of acute traumatic syringomyelia with multiple cavitations of the axis-cylinders of the spinal cord is dependent on the liquid transmission of pressure pulses from the high to the low pressure area by the sudden crushing blow on the central nervous system. The excessive lateral expansions with 'blow outs' of the axis-cylinders by the pressure of mechanical trauma or strychnine stimulation are the result of the liquid transmission of compression. When a nerve is sectioned and stimulated by the faradic current, liquid droplets of the axis-cylinders are expressed from the cut end by the compression pulses of the electric current. The transmission of the

nerve impulse tends to produce multiple lateral expansions of the liquid axis-cylinders which conduct the pressure pulses."

The nerve impulse appears to be transmitted through the entire cross-sectional area of the axis cylinder, which may vary in a reversible manner either partially or completely from a liquid to a semiliquid state. In view of the objective evidence herein presented, neurofibrils can hardly be thought to remain the specific structural conductors of nerve action. The neurofibrils ought rather to be considered as longitudinally condensed parts of the colloidal liquid of the axon drawn out into a microcapillary filament by the tension of relative growth and the streamline effect of metabolic activity and axonic flow. The neurofibrils are inconstant structures; they vary with the chemical condition and physical state and shape of the axis cylinder; they are the physical resultants of longitudinal tension and lateral compression in an extended protoplasmic strand. Comparable physical and chemical conditions form myofibrils in muscle.

Jackson,[55] in his study of visual fatigue, concluded with the following pertinent statements in reference to the alterations in the neuromuscular apparatus in fatigue.

"The number of points at which fatigue might occur so as to lower activity or cause sensations of which we are conscious, are thus enumerated by Herrick,[56] who bases them on the summary of Stiles: [57] (1) fatigue of muscle fibres, (2) fatigue of the junction of the motor nerve with the muscle fibre at the motor end-plate, (3) fatigue of the nerve-fibres, (4) fatigue of the motor nerve cells, (5) of the synapses between the nerve cells, (6) fatigue of the sense organs and afferent apparatus, (7) fatigue of the centers of voluntary control.

"Physiologic experiment shows that the nerve fibres are capable of conducting impulses after the neuro-muscular apparatus has become exhausted and fails to respond to stimulus; and that muscle fibres cease to respond to impulses coming thru the nerve trunk, and yet contract well under direct stimulation. This shows that neither the muscles or the nerve fibre is exhausted.

"Stiles [57] thinks the junction of nerve and muscle is especially likely to give out under continued stimulation. He says: 'One is tempted to draw a comparison between the end-plate and the safety fuse such as is used in connection with an electric fixture. The fuse is intended to be destroyed under conditions which might otherwise threaten damage to more valuable portions of the system. It is readily renewed. So we may think of the end-plate as something easily impaired by use, but also easy to repair. It is better that wear and tear should fall upon this structure than upon the more highly organized protoplasm of nerve cells or muscle fibres.' "

These concepts are supported by the morphologic findings reported in this paper. In fact, before our attention was drawn to the similar analogy previously made by Stiles,[57] we had designated the motor end-plates as chemical safety fuses, basing our designation on facts observed in our lactic acid series of experiments.

The variable time factor involved in the production of the localized and irregular morphologic changes of axonorrhea, as well as the final

disappearance of the motor end-plates in certain parts of different muscles, may account for the conflicting reports on the pathologic function of the neuromuscular apparatus in shock patients quoted by Cannon [9] (pages 1, 2, 3, and 119 respectively):

"If the limbs are lifted and then let go, they immediately fall as if dead. ... On being thus questioned he complains of cold, faintness, and deadness of the extremities." (Fischer.[58])

"The muscles are not paralyzed anywhere, but the patient seems disinclined to make any muscular effort." (Warren.[59])

"Thus Mitchell, Morehouse, and Keen,[60] in their report to the Surgeon General of the United States Army in 1864, described cases occurring after wounds of grave nature, in which the patient, immediately after being wounded, suffered from a state of depression which continued and which was marked by great weakness, feeble circulation, pallor, etc."

"Cowell [61] reported no reduction of the strength of grip in shock and he observed one wounded man, pulseless and showing a systolic pressure of approximately 30 mm. Hg, who was so vigorous in his movements that two orderlies were required to hold him on the stretcher."

Observations by Brown-Séquard [62] demonstrated that the intravascular injection of alkaline solutions in animals and of defibrinated blood in a decapitated criminal removed rigor and re-established muscle irritability; by Wedensky,[63] that indirect overstimulation of muscle through its nerve supply led to inhibition of transmission of the nerve impulse at the motor end-plates; by Wells,[64] that lactic acid caused Zenker's waxy degeneration of muscle; by Norris,[65] that the time of onset of rigor was influenced by the intact peripheral nerves; by Matthews,[66] that occlusion of circulation and muscle activity produced pain and "explosive" changes in the electric potential of the hyperexcitable sensory spindles; by Gesell and his co-workers,[7] that increased hydrogen ion concentration in muscle resulted in the discharge of augmented amounts of acetylcholine at the motor end-plates; and by Rosenbohm,[67] that nerve cell proteolysis was produced by lactic acid.

Morphologic evidence, therefore, is being accumulated that supports the conclusion that normal metabolism maintains, and abnormal metabolism alters, the structure of the motor end-plates in skeletal muscle in health and disease. There is, furthermore, morphologic evidence, clearly demonstrable by the methods of experimental pathology, that the motor end-plates are microscopic endocrine glands that pour their secretion directly into and between the muscle fibers. This supports the chemical theory of impulse transmission from nerve to muscle in the maintenance of normal muscle tonus, contraction, relaxation, and heat production. The hypersecretion of axonal substance into certain pathologic muscle fibers and the denervation at the myoneural junction in shock may be the abnormal changes that underlie the hyperexcitability, spasm, changes in heat production, and flaccid palsy caused by

altered muscle metabolism. These liberate augmented amounts of toxic metabolites under conditions of anoxia associated with overstimulation resulting in structural nervous exhaustion of the neuromuscular apparatus and acute muscular degeneration in localizing areas. A microscopic survey of entire muscles must be made, therefore, before a pathologist is warranted to express an opinion whether muscles are normal or abnormal in either shock or poliomyelitis. There is no short cut by inspection of a few sections of muscle tissue.

Human and experimental poliomyelitis produces alterations of the neuromuscular apparatus very similar to those resulting from the local injection experimentally of lactic acid and to those of shock (hemorrhagic, thermal, chemical, infectious, and traumatic). Whether the changes of the motor end-plates in poliomyelitis are primary or secondary is still undetermined. There is no doubt that the changes produced locally by the injection of lactic acid in the muscle are primary. In the early stages of shock there is considerably greater alteration detected at the myoneural junction than in the cell bodies of the spinal cord. In the late stages of shock the ventral horn cells of the spinal cord likewise show pathologic changes but not to the same degree of dissolution as is found in the motor end-plates.

These changes of the motor end-plates, therefore, are not peculiar to shock nor characteristic for it alone; they are essentially fundamental and capable of general application in many disease processes. The changes may be regarded as the manifestations of functional activity carried beyond normal limits to its termination in fatigue, spasm, flaccid palsy, and structural exhaustion. The sequence of these structural changes is: (1) an increase of the gold-chromatic material (hyperchrysophilia) in the motor end-plate; (2) axonorrhea or the discharge of abnormal quantities of the hyperchrysophilous material from motor end-plates increased in permeability; (3) a progressive decrease to the point of complete disappearance of the gold-chromatic material (hypochrysophilia) in both the hypolemmal and epilemmal axons; and (4) the evanescent appearance of extra-axonic gold-chromatic material in the muscle fibers.

The quantitative increase of the gold-chromatic material in the axons in shock is absolute in comparison to normal changes in the motor end-plates. The actual increase in the gold-chromatic material results from an augmentation of the axonic substance in the motor end-plates which finally are increased in permeability. Under conditions of shock the ordinary granules of Kühne discharged into the muscle fuse into larger masses. These masses of gold chromatic substances are found close to, as well as at a considerable distance away from, the motor end-plates.

These facts are morphologic evidence which supports the conclusion that the motor end-plates are microscopic glands of internal secretion. The essence of this secretory process in shock is the continued overproduction of gold-chromatic material followed by its dissolution to the point of complete structural disappearance of the motor end-plates in skeletal muscle. These morphologic changes may be produced suddenly and with explosive violence or they may be gradual in onset dependent upon the intensity and duration of the abnormal shock stimulus to, and the resistance of, the living organism, especially the living nervous, muscular and vascular systems.

Summary

1. Whole-mount specimens of skeletal muscle and motor end-plates were studied by the gold-and-teasing method under low and high powers of the microscope in the gastrocnemius muscles from 100 unanesthetized white rats traumatized in the Noble-Collip drum. The changes of the motor end-plates in the 200 gastrocnemius muscles of the traumatized animals were compared with 20 relatively normal control muscles excised from 10 animals 15 minutes after the intraperitoneal injection of pentobarbital sodium.

2. There was a sequence of overlapping changes produced by trauma on the neuromuscular apparatus:

(a) The first effect of traumatic shock was hyperchrysophilia (increased intensity of gold impregnation) of the motor end-plates.

(b) The second effect of traumatic shock was an axonorrhea (abnormal discharge from the permeable end-plates) of hyperchrysophilous axonic material into and among the muscle fibers.

(c) The third effect of traumatic shock was a progressive hypochrysophilia leading to disappearance of the motor end-plates due to structural exhaustion. The increased permeability of the motor end-plates produced an abnormal discharge of the chrysophilous material of the axons into the muscle to the point of complete depletion of the axonic structure of the motor end-plates.

3. The extra-axonic material secreted from the motor end-plates into the muscle is an ephemeral and tenuous liquid that forms masses in the muscle fiber, which masses were either homogeneous or cross-striated. This secreted nervous material was found in greatest abundance within 20 minutes after the rats had made 800 revolutions in the Noble-Collip drum rotating at the rate of 40 revolutions per minute. This chrysophilous material secreted by the axons of the motor end-plates disappeared from the myoplasm of the muscle fiber within 1 hour after the rats made 800 revolutions in the drum.

4. The increased quantity of extra-axonic material discharged from the motor end-plates was accompanied in some muscle fibers by an acute granulation, liquefaction, and hyaline degeneration of the myoplasm with loss of the cross striations. Large quantities of the gold-impregnated muscle must be surveyed before a reliable opinion may be rendered regarding the presence or absence of pathologic changes in a specific muscle.

5. It is suggested that overstimulation due to overactivity and trauma of the white rats in the Noble-Collip drum produces increased secretion of the axonic liquid of the motor end-plates into muscle. This results in acute degeneration of some of the muscle fibers. It had been demonstrated previously that increasing the quantity of lactic acid in the muscle destroyed the motor end-plates. Overactivity, likewise, increases the quantity of metabolites, including lactic acid, in the muscle, and produces localized dilatation of the small blood vessels, and causes local perivascular edema. Thus, localized muscle anoxia is produced. Two factors are at work in the structural exhaustion of the motor end-plates: (1) overstimulation of the nervous system, and (2) localized anoxia of the muscle fibers and accumulation of lactic acid, keto-acid metabolites, and other products of anerobic metabolism.

6. The morphologic depletion of the motor end-plates and acute degeneration of certain skeletal muscle fibers are structural expressions of a profound alteration in the underlying biochemical processes of the neuromuscular apparatus. The neuromuscular junction may be one site where toxins are produced. The pathologic changes which occur at the neuromuscular apparatus may be one of the factors in the trigger mechanism which initiates traumatic shock. This initiation may be sudden or slow depending upon the strength and duration of the stimulus, the resistance of the living organism, and the degree of pathologic change in the neuromuscular apparatus. In irreversible shock with flaccid paralysis there is denervation of the muscle at the myoneural junction. The pathologic state of the muscle in shocked rats is highly variable: ante-mortem rigidity, continuous or periodic spasm, fasciculation, or flaccid paralysis may occur. Most of the motor end-plates had disappeared from the tree of innervation in those muscles that failed to respond to both direct and indirect electric or mechanical stimulation. There was a correlation, therefore, between flaccid muscular paralysis and peripheral nervous exhaustion of the structure at the myoneural junction. In some animals the muscles failed to respond to indirect, but did respond to direct electric stimulation. Under such conditions the motor end-plates were either abnormally retracted into ball-like structures or were undergoing acute degeneration.

7. Conclusive objective morphologic evidence, confirming our previous studies, is presented to sustain the theory that the motor end-plates are microscopic glands of internal secretion. This anatomic fact confirms the physiologic and chemical findings supporting the chemical theory of the transmission of the nerve impulse.

8. The degree of pathologic change in the motor end-plates is greater than that of the cell bodies of the spinal cord in rats shocked in the Noble-Collip drum.

9. The sensory axons of the muscle and tendon spindles have dilatations indicative of overstimulation.

10. The anatomic changes in the neuromuscular apparatus produced by traumatic, thermal, and hemorrhagic shock are similar to those found in experimental and human poliomyelitis and in certain other diseases in man.

11. Because of the fact that the motor end-plates disappear quickly during post-mortem anoxia and rigor mortis, they must be studied as soon as possible after death and preferably from muscles excised prior to death, and in man from specimens taken for biopsy. However, ante-mortem changes of the motor end-plates accompanying certain morbid processes of the vascular and nervous systems are quite comparable to those which occur during the post-mortem survival period and lead to complete autolysis of the end-plates. This ante-mortem autolysis of end-plates and the localized acute changes in the muscles may be two of the many factors that underlie the malaise, muscle weakness, and paralysis which accompany certain acute infectious processes with toxemia.

12. In evaluating the variable pathologic function and structure of the neuromuscular apparatus in traumatic shock, the factors of intensity and duration of the stimulus, of the time after trauma, and of individual variations of resistance of the living organism must be considered. Fatal traumatic shock is the culmination of a complex sequence of events in the pathologic structure and functioning of the living organism that may be sudden or slow in onset. When traumatic shock is induced in unanesthetized rats in the Noble-Collip drum there are profound pathologic changes in the peripheral nervous system manifested at the neuromuscular apparatus which heretofore have not been studied.

13. It is suggested that the therapeutic agents used in traumatic shock; namely, debridement, blood plasma, and certain supportive measures, are amply sustained by the pathologic findings of the neuromuscular apparatus and localized packing of blood cells in, and plasma loss from, the small vessels of certain muscles. Probably a study of the

agents that would specifically neutralize the hypersecretion from the motor end-plates during the initial stages of shock would render therapy still more effective than at the present time. The application of certain aspects of the therapy of shock to similar conditions produced by infections, namely, poliomyelitis, warrants experimental investigation.

14. Finally, the evidence suggests that any concept of muscle structure which assumes constancy in the number of so-called fixed sarcomeres composed of rigid membranes is a pure morphologic myth handed down by certain histologists for over 100 years. The cross striations are structural expressions that vary in thickness and in number with the variable chemical composition and concentration of the microcapillary muscle fiber. In other words, the variable cross striations are morphologic resultants of the different changes in muscle metabolism.

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Addendum

Subsequent to the acceptance of this paper for publication, the term *neurosomes* has been used to designate the gold-impregnated inclusion masses discharged from the motor end-plates into and between muscle fibers during thermal shock of rats [68] and chameleons.

REFERENCES

1. Carey, E. J. Experimental pleomorphism of motor nerve plates as a mode of functional protoplasmic movement. *Anat. Rec.*, 1941, **81**, 393–413. Studies on ameboid motion and secretion of motor end-plates. III. Experimental histopathology of motor end-plates produced by quinine, curare, prostigmine, acetylcholine, strychnine, tetraethyl lead and heat. *Am. J. Path.*, 1944, **20**, 341–393. Morphologic effects of poliomyelitis virus upon motor end-plates in the monkey. *Proc. Soc. Exper. Biol. & Med.*, 1943, **53**, 3–5. Studies on ameboid motion and secretion of motor end-plates. IV. Anatomic effects of poliomyelitis on the neuromuscular mechanism in the monkey. *Am. J. Path.*, 1944, **20**, 961–995. Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E. Anatomic changes of motor nerve endings in human muscles in early poliomyelitis. *J. Neuropath. & Exper. Neurol.*, 1944, **3**, 121–130.

2. Carey, E. J., and Massopust, L. Sudden destruction of motor end-plates by lactic acid. *Proc. Soc. Exper. Biol. & Med.*, 1944, **55**, 194–197.

3. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J. Acute anatomic breakdown of motor end-plates in hemorrhagic shock. *Proc. Soc. Exper. Biol. & Med.*, 1944, **56**, 115–118.

4. Govier, W. M., and Greer, C. M. Studies on shock induced by hemorrhage. II. Effect of thiamin on disturbances of carbohydrate metabolism. *J. Pharmacol. & Exper. Therap.*, 1941, **72**, 321–330.

5. Gutmann, H., Olson, W. H., Kroll, H. H., Levinson, S. O., and Necheles, H. Chemical studies in traumatic shock. *Am. J. Physiol.*, 1941, **133**, P308-P309.
6. Cole, W. H., Allison, J. B., Leathem, J. H., Nastuk, W. L., and Anderson, J. A. Acidosis in the rabbit during "gravity shock." *Anat. Rec.*, 1942, **84**, 468.
7. Gesell, R., Brassfield, C. R., and Hansen, E. T. Possible rôles of cH in neurophysiology. *Proc. Fed. Am. Socs. Exper. Biol.*, 1942, **1**, 29. Hansen, E. T., Worzniak, J. J., and Gesell, R. Physiological effects of artificially administered acetylcholine and eserine. *Ibid.*, 1942, **1**, 36.
8. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J. Studies on ameboid motion and secretion of motor end-plates. V. Experimental pathologic effects of traumatic shock on motor end-plates in skeletal muscle. *J. Neuropath. & Exper. Neurol.* 1945, **4**, 134–145.
9. Cannon, W. B. Traumatic Shock. D. Appleton and Co., New York, 1923.
10. Moon, V. H. Shock and Related Capillary Phenomena. Oxford Univ. Press, New York, 1938.
11. Kühne, W. Neue Untersuchungen über motorische Nervenendigung. *Ztschr. f. Biol.*, 1887, **23**, 1–148.
12. Bremer, J. L. A Text-Book of Histology Arranged Upon an Embryological Basis. Blakiston Co., Philadelphia, 1936, ed. 5, p. 166.
13. Noble, R. L., and Collip, J. B. A quantitative method for the production of experimental traumatic shock without haemorrhage in unanaesthetized animals. *Quart. J. Exper. Physiol.*, 1941–42, **31**, 187–199.
14. Carey, E. J. Studies on ameboid motion of motor nerve plates. II. Pathologic effects of CO_2 and electricity on the explosive ameboid motion in motor nerve plates in intercostal muscle. *Am. J. Path.*, 1942, **18**, 237–289.
15. Boeke, J. The innervation of striped muscle-fibres and Langley's receptive substance. *Brain*, 1921, **44**, 1–22.
16. Huber, G. C. A note on sensory nerve-endings in the extrinsic eye-muscles of the rabbit. "Atypical motor-endings" of Retzius. *Anat. Anz.*, 1899, **15**, 335–342.
17. Huber, G. C., and DeWitt, L. M. A. A contribution on the motor nerve-endings and on the nerve-endings in the muscle-spindles. *J. Comp. Neurol.*, 1897–98, **7**, 169–230.
18. Wilkinson, H. J. The innervation of striated muscle. *M. J. Australia*, 1929, **2**, 768–793. Experimental studies on the innervation of striated muscle. *J. Comp. Neurol.*, 1930, **51**, 129–151.
19. Fletcher, W. M., and Hopkins, F. G. Lactic acid in amphibian muscle. *J. Physiol.*, 1906–07, **35**, 247–309.
20. DuBois-Reymond, E. Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysik. Veit & Co., Leipzig, 1877, **2**, 700.
21. Langley, J. N. On nerve endings and on special excitable substances in cells. *Proc. Roy. Soc., London, s. B.*, 1906, **78**, 170–194.
22. Loewi, O. Über humorale Übertragbarkeit der Herznervenwirkung. Part I. *Pflüger's Arch. f. d. ges. Physiol.*, 1921, **189**, 239–242. Über humorale Übertragbarkeit der Herznervenwirkung. Part II. *Ibid.*, 1921–22, **193**, 201–213. The humoral transmission of nervous impulse. *Harvey Lectures*, 1932–33, **28**, 218–233.
23. Dale, H. H., and Feldberg, W. The chemical transmitter of vagus effects to the stomach. *J. Physiol.*, 1934, **81**, 320–334.
24. Cannon, W. B. Chemical mediators of autonomic nerve impulses. *Science*, 1933, **78**, 43–48.

25. Gesell, R., Brassfield, C. R., Hansen, E. T., and Mason, A. Excessive acidity of the nerves. *Science,* 1942, **95** (suppl. Apr. 10, 1942), 11.
26. Mitchell, S. W. Injuries of Nerves and Their Consequences. J. B. Lippincott & Co., Philadelphia, 1872.
27. Mitchell, S. W. Paralysis from peripheral irritation with report of cases. *New York Med. J.,* 1866, **2,** 321–355.
28. Green, H. N. Shock-producing factors from striated muscle; isolation and biological properties. *Lancet,* 1943, **2,** 147–153. Bielschowsky, M., and Green, H. N. Shock-producing factors from striated muscle; fractionation, chemical properties and effective doses. *Ibid.,* 1943, **2,** 153–155.
29. Dale, H. H. Conditions which are conducive to the production of shock by histamine. *Brit. J. Exper. Path.,* 1920, **1,** 103–114.
30. Cannon, W. B. Acidosis in cases of shock, haemorrhage, and gas infection. *Medical Research Council, Special Report Series, No. 25,* His Majesty's Stationery Office, London, 1919, pp. 85–98.
31. Bayliss, W. M. Gum injections in "shock." *J. Physiol.,* 1918–19, **52,** xvii–xviii.
32. Blalock, A. Experimental shock. VII. The importance of the local loss of fluid in the production of the low blood pressure after burns. *Arch. Surg.,* 1931, **22,** 610–616.
33. Harkins, H. N. Recent advances in the study and management of traumatic shock. *Surgery,* 1941, **9,** 231–294; 447–482; 607–655.
34. Freeman, N. E., Freedman, H., and Miller, C. C. Production of shock by prolonged continuous injection of adrenalin in unanesthetized dogs. *Am. J. Physiol.,* 1941, **131,** 545–553.
35. Remak, R. Ueber den Inhalt der Nervenprimitivröhren. *Arch. f. Anat., Physiol. u. wissensch. Med.,* Berl., 1843, 197–201.
36. Schultze, M. In: Stricker, S. Handbuch der Lehre von den Geweben. W. Engelmann, Leipzig, 1871, p. 108.
37. de Rényi, G. S. The structure of cells in tissues as revealed by microdissection. II. The physical properties of the living axis cylinder in the myelinated nerve fiber of the frog. *J. Comp. Neurol.,* 1928–29, **47,** 405–425.
38. Parker, G. H. The neurofibril hypothesis. *Quart. Rev. Biol.,* 1929, **4,** 155–178.
39. Verworn, M. General Physiology. Macmillan & Co., New York, 1899, p. 581.
40. von Lenhossék, M. Ueber die physiologische Bedeutung der Neurofibrillen. *Anat. Anz.,* 1910, **36,** 257–281.
41. Koltzoff, N. K. Zur Frage der Zellgestalt. *Anat. Anz.,* 1912, **41,** 183–207.
42. Goldschmidt, R. In: Festschrift zum 60. Geburtstage Richard Hertwigs. G. Fischer, Jena, 1910, **2,** p. 253.
43. von Szüts, A. Zur mechanischen Morphologie der Nervenelemente. *Anat. Anz.,* 1914–15, **47,** 199–201.
44. Jenkins, O. P., and Carlson, A. J. The rate of the nervous impulse in the ventral nerve-cord of certain worms. *J. Comp. Neurol.,* 1903, **13,** 259–289.
45. Carlson, A. J. Further evidence of the fluidity of the conducting substance in nerve. *Am. J. Physiol.,* 1905, **13,** 351–357.
46. Meek, W. J., and Leaper, W. E. Effects of pressure on conductivity in nerve and muscle. *Am. J. Physiol.,* 1910–11, **27,** 308–322.
47. Lapicque, L., and Legendre, R. Relation entre le diamètre des fibres nerveuses et leur rapidité fonctionnelle. *Compt. rend. Acad. d. sc.,* 1913, **157,** 1163–1166.
48. Erlanger, J. The interpretation of the action potential in cutaneous and muscle nerves. *Am. J. Physiol.,* 1927, **82,** 644–655.

49. Erlanger, J., and Gasser, H. S. Electrical Signs of Nervous Activity. University of Pennsylvania Press, Philadelphia, 1937, p. 130.

50. Gasser, H. S., and Erlanger, J. The rôle played by the sizes of the constituent fibers of a nerve trunk in determining the form of its action potential wave. *Am. J. Physiol.*, 1927, **80**, 522–547.

51. Young, J. Z. Structure of the sheaths of Maia nerve fibres. *J. Physiol.*, 1935, **85**, 2P–3P.

52. Gerard, R. W. Nerve conduction in relation to nerve structure. *Quart. Rev. Biol.*, 1931, **6**, 59–83.

53. Weiss, P. Damming of axoplasm in constricted nerve: a sign of perpetual growth in nerve fibers. *Anat. Rec.*, 1944, **88**, 464.

54. Carey, E. J. Wave mechanics of protoplasmic action. XII. Experimental acute traumatic syringomyelia. *Arch. Path.*, 1937, **24**, 419–429.

55. Jackson, E. Visual fatigue. *Am. J. Ophth.*, 1921, s. 3, **4**, 119–122.

56. Herrick, C. J. An Introduction to Neurology. W. B. Saunders Co., Philadelphia & London, 1915.

57. Stiles, P. G. The Nervous System and Its Conservation. W. B. Saunders Co., Philadelphia & London, 1914.

58. Fischer, H. Ueber den Shok. *Samml. klin. Vortr.*, 1870–75, no. 10 (Chirurgie, no. 5), 69–82.

59. Warren, J. C. Surgical Pathology and Therapeutics. W. B. Saunders Co., Philadelphia, 1895.

60. Mitchell, S. W., Morehouse, G. R., and Keen, W. W., Jr. Reflex paralysis, the result of gunshot wounds, founded chiefly upon cases observed in the United States General Hospital. Report to the Surgeon General, 1864, Circular No. 6.

61. Cowell, E. M. The initiation of wound shock. *Medical Research Council, Special Report Series, No. 25*, His Majesty's Stationery Office, London, 1919, pp. 99–108.

62. Brown-Séquard, E. Apparition de la rigidité cadavérique avant la cessation des battements du coeur. *Compt. rend. Soc. de biol.*, 1850, **2**, 194–195. Nouveaux faits relatifs à la rigidité cadavérique. *Compt. rend. Soc. de biol.*, 1885, s. 8, **2**, 249–250. Des contractions et des élongations en apparences spontanées des muscles atteints de la rigidité cadavérique. *Arch. de physiol. norm. et path.*, 1889, s. 5, **1**, 675–682. Sur des actions inconnues ou à peine connues des muscles après la mort. *Arch. de physiol. norm. et path.*, 1889, s. 5, **1**, 726–732.

63. Wedensky, N. E. Die Erregung, Hemmung und Narkose. *Arch. f. d. ges. Physiol.*, 1903–04, **100**, 1–144.

64. Wells, H. G. The pathogenesis of waxy degeneration of striated muscles (Zenker's degeneration). *J. Exper. Med.*, 1909, **11**, 1–9.

65. Norris, R. On the nature of rigor mortis. *J. Anat. & Physiol.*, 1866–67, **1**, 114–119.

66. Matthews, B. H. C. Nerve endings in mammalian muscle. *J. Physiol.*, 1933, **78**, 1–53.

67. Rosenbohm, A. Die Spaltungsprodukte des Glutathions im lebenden Gewebe und die Beziehung des Glutathions zum proteolytischen Abbau bei der Ausbreitung von Krebsgeschwülsten. *Biochem. Ztschr.*, 1937, **289**, 279–287.

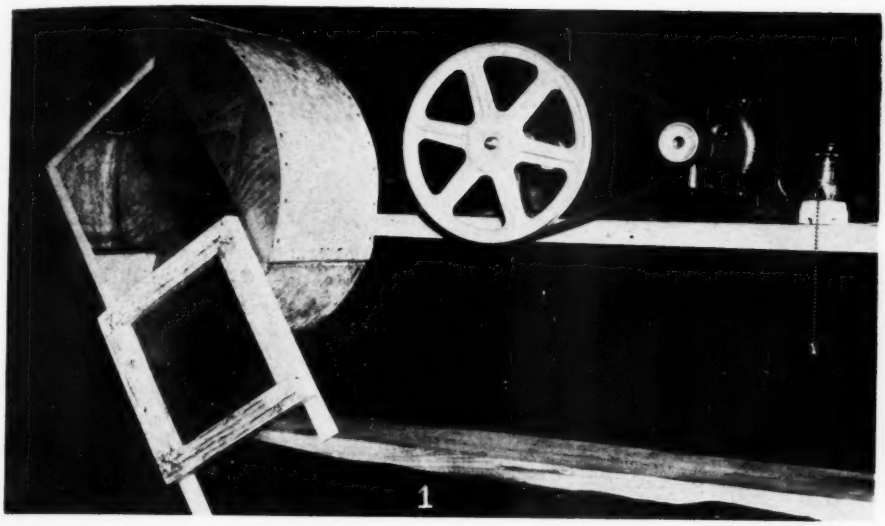
68. Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E. Experimental discharge of neurosomes into spastic muscles of rats by thermal shock. *Anat. Rec.*, 1945, **91**, 268–269.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 157

FIGS. 1 and 2. The lateral and upper aspects of the motor-driven Noble-Collip drum. There is a single, circular, galvanized iron drum borne by a short, externally fixed central shaft running on ball bearings. On this shaft are wheels of various size for the driving belt. The drum has an inside diameter of 15 inches and a depth of 8 inches. The two projections on the inside of the drum are blunt, triangular in shape on cross section, with a height of 2 inches and a base of 3 inches. The outside of the drum has a hinged window, part of which is covered by heavy cellophane so that observations may be made.



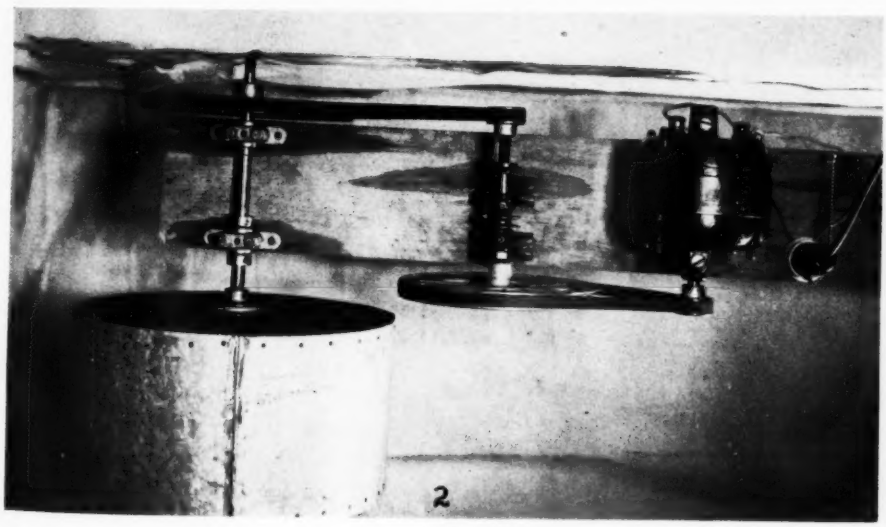


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PLATE 158

The photomicrographs of Plates 158 to 178 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat (*Mus norvegicus*), previously prepared by the gold technic. The photographs were direct contact prints from the negatives which were exposed through the microscope and not subjected to subsequent enlargement. They may, therefore, be compared with those of the white rat and the chameleon previously published. In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; and "Kg.," masses of extra-axonic Kühne's granules. There has been no retouching of either negatives or prints. Traumatic shock was produced in unanesthetized rats in the Noble-Collip drum. Unless otherwise specified, each animal was subjected to 800 revolutions at the rate of 40 r.p.m.

FIG. 3. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 5 minutes after the rat had been subjected to trauma. Some of the end-plates are replaced by fine granules and others are completely absent. The epilemmal axons in certain places, therefore, are denuded of their hypolemmal nerve terminals. In some locations elongated streamers of black, gold-staining substance are found. This material accumulates both within and without the muscle fibers during the period that the motor innervation is depleted of its gold-staining substance. This streamer, Kg., has been broken into segments. × 125.

FIG. 4. Sprays of medullated nerve fibers and motor end-plates of variable size from a relatively normal gastrocnemius muscle from a rat of the control series subjected for 15 minutes to nembutal anesthesia. It is necessary for animals used as relatively normal controls to designate either the method of anesthesia or the mechanical method of death such as bleeding or decapitation, because of the high degree of morphologic sensitivity of the motor-end plates to any physical or chemical agent. × 125.

FIG. 5. Sprays of medullated nerve fibers, motor end-plates, and dark masses of axonic material from the gastrocnemius muscle 5 minutes after it had been subjected to trauma. Some of the end-plates are absent, others are granular and in direct relationship with certain dark-staining masses of extruded axonic material. × 125.

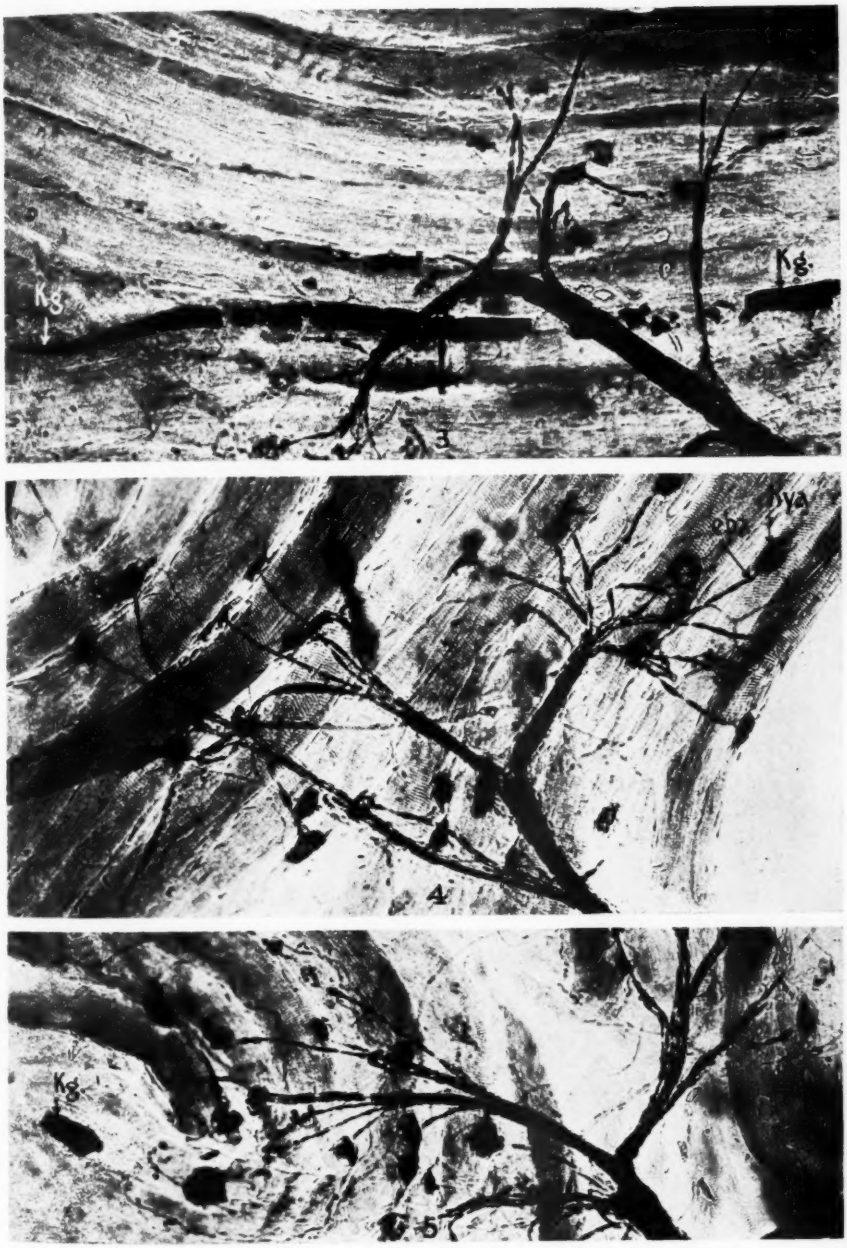

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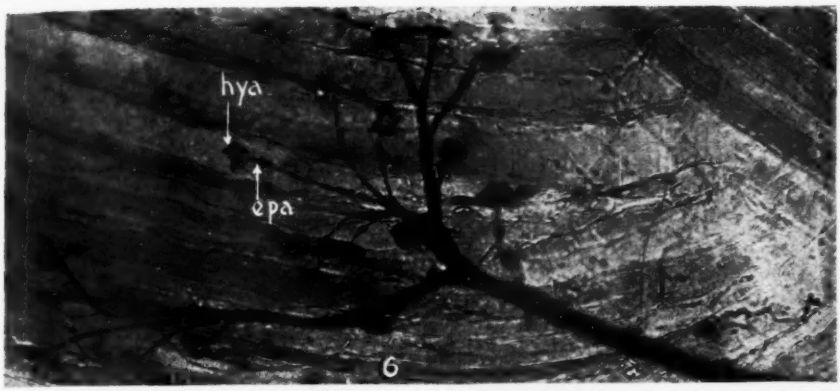
PLATE 159

FIGS. 6 and 7. Sprays of medullated nerve fibers and motor end-plates showing variable degrees of projection of axonic material into the muscle 5 minutes subsequent to trauma. In some places there is a fine filament between the epilemmal and hypolemmal axon. In others there is a small droplet at this location. Some of the end-plates are retracted into dark-staining masses and others are expanded. Elongated streamers of projected axonic material (Kg.), which represent one stage in the loss of motor innervation, are clearly evident (Fig. 7) in direct continuity with the altered motor end-plates. × 125.

FIG. 8. Sprays of medullated nerve fibers of the gastrocnemius muscle completely denuded of their hypolemmal axons 1 hour after the muscle had been subjected to trauma. The liquefied motor end-plates are identified in some locations by the fine granular remains of the plate, while in others no evidence of the dissolved end-plate is observed. In places the related cross striations have likewise undergone a granular alteration and liquefaction. In certain locations the epilemmal axon terminates in a sharp point and in others in a rounded knob. The change in the motor innervation of the traumatized muscle begins distad at the motor end-plate and progresses in a centripetal direction in the epilemmal axons. The granular degeneration, beading, and fragmentation of the epilemmal axon therefore extend in a central direction and away from the altered end-plates after muscle trauma. × 125.



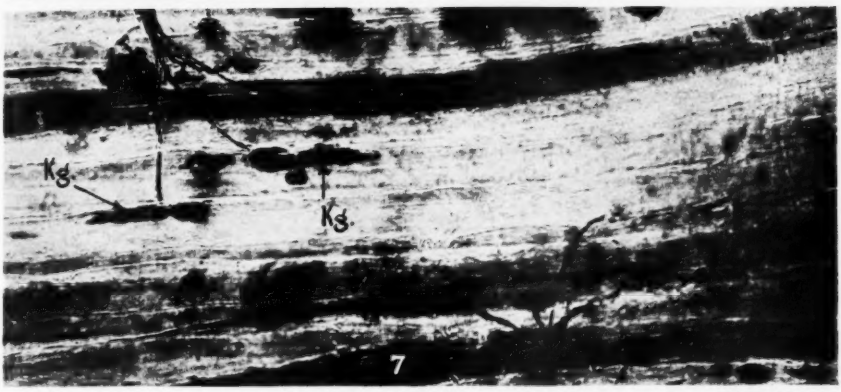


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PLATE 160

FIGS. 9 to 12. Sprays of medullated nerve fibers and motor end-plates of variable sizes in the gastrocnemius muscle 15 minutes after the animal had been subjected to trauma. Dark-staining oval droplets and elongated streamers of axonic material are in direct relation to the traumatized end-plates in many locations. Some end-plates are retracted, others are expanded, and still others are completely liquefied. The normal granular sole of Kühne is replaced in some locations by an abnormal accumulation of the axonic material both within and without the motor end-plate. In some locations the muscle fibers have the histologic characteristics of Zenker's waxy degeneration. × 125.

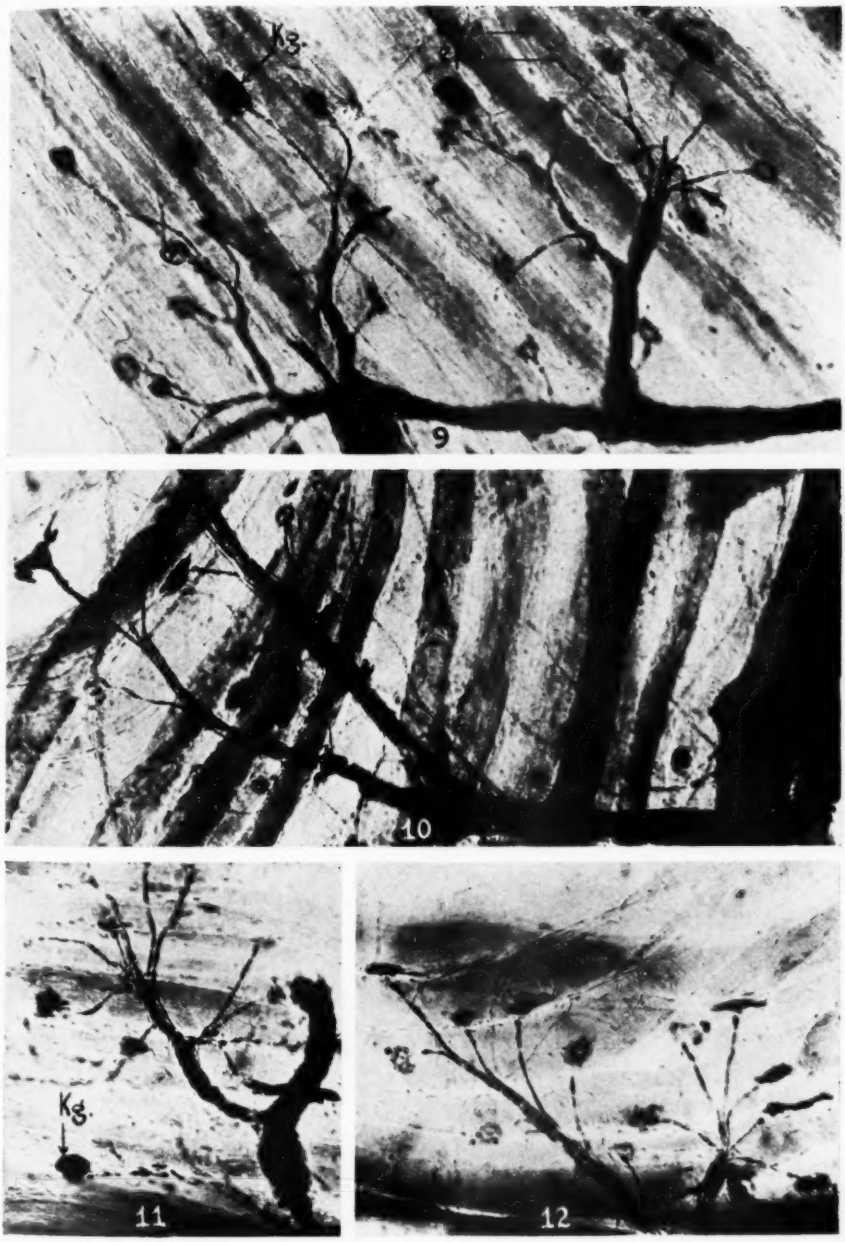

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PLATE 161

FIGS. 13 and 14. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes after the animal was traumatized in the rotary drum. The axonic droplets are found both within and without the motor end-plates. In some plates this axonic material is found in direct continuity with the motor end-plate, and in others separated from the end-plate and projected into the muscle fiber (Fig. 13). These axonic streamers vary from 10 to 150 μ in length and from 2 to 50 μ in diameter. The axonic droplets are unipolar, bipolar, and irregular in arrangement in relation to the highly permeable motor end-plate. Waxy degeneration of the muscle fibers is evident in some locations. $\times$ 125.

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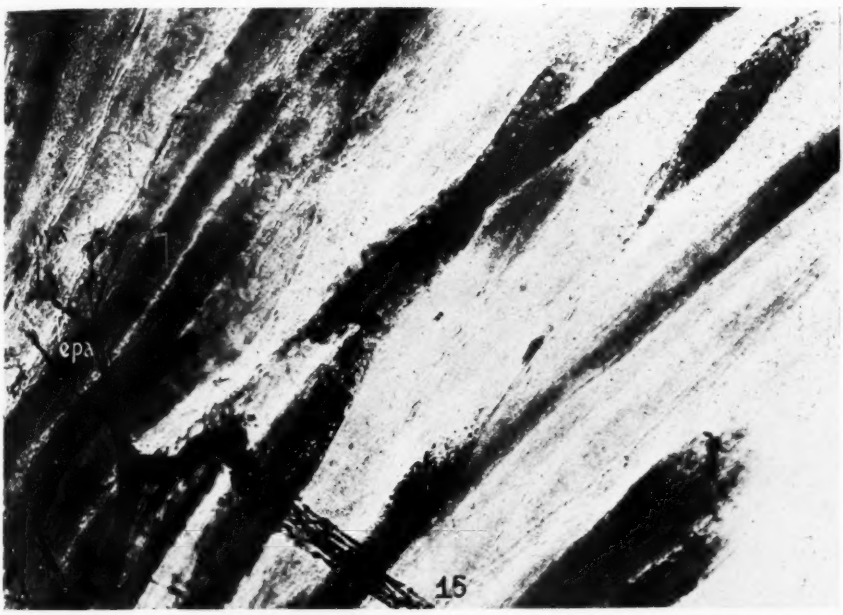
PLATE 162

FIGS. 15, 16, and 17. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization in the rotary drum. There are variable degrees of loss of motor innervation by the abnormal pouring out of axonic material both within and without the muscle fibers. The end-plates are either expanded or retracted, and in some locations completely absent. Progressive steps in the transmission of the dark-staining axonic material may be traced through the epilemmal axon, hypolemmal axon, and external to the end-plate into the muscle fiber. Projected axonic material is either unipolar or bipolar in arrangement, or irregular in its disposition in relation to the motor end-plate. Some of the traumatized muscle fibers (Fig. 15) form fusiform structures and exhibit an alteration of the myoplasm similar to that found in waxy degeneration produced by lactic acid. × 125.



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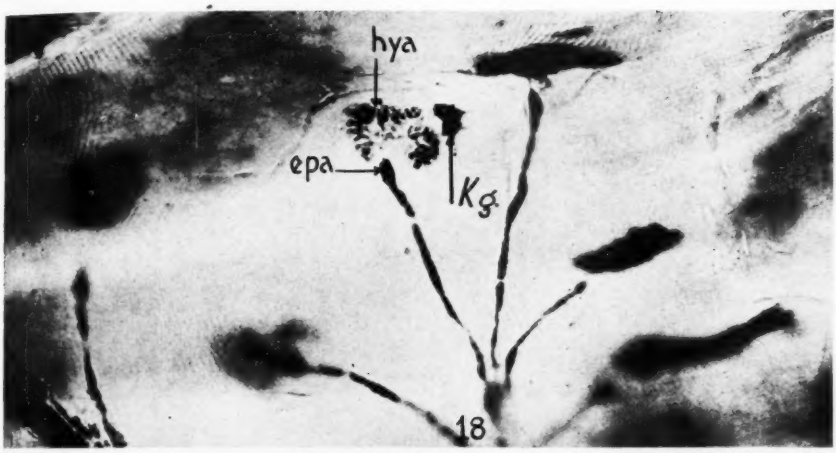
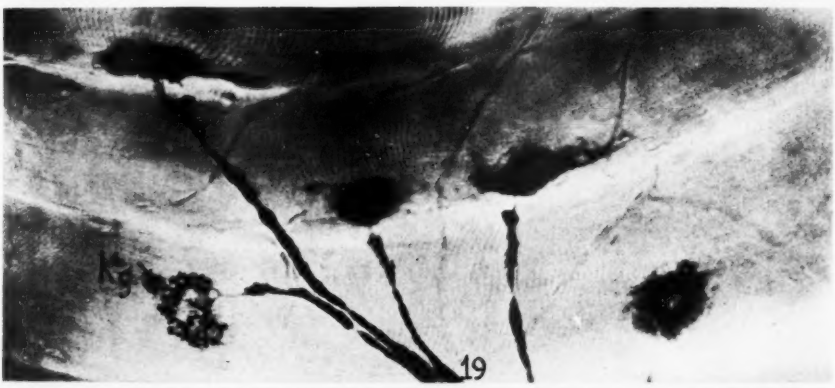
Neuromuscular Apparatus and Traumatic Shock

PLATE 163

FIGS. 18, 19, and 20. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization in the rotary drum. There is an irregular dilatation and constriction of the epilemmal axons (epa). The hypolemmal axons (hya) are either retracted or expanded. Some of the end-plates (Fig. 19) have an accumulation of axonic material in the center of the plate which forms a hub-like appearance. In the expanded plates there is a diminution of the granular sole of Kühne which is replaced by a unipolar droplet of this granular material (Figs. 18 and 19, Kg.). In other locations the end-plate has a large, oval-shaped droplet of the projected axonic material (Fig. 20, Kg.). × 350.





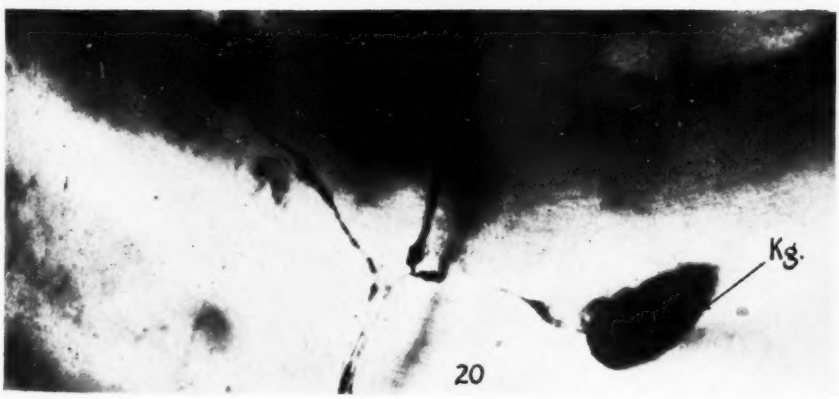


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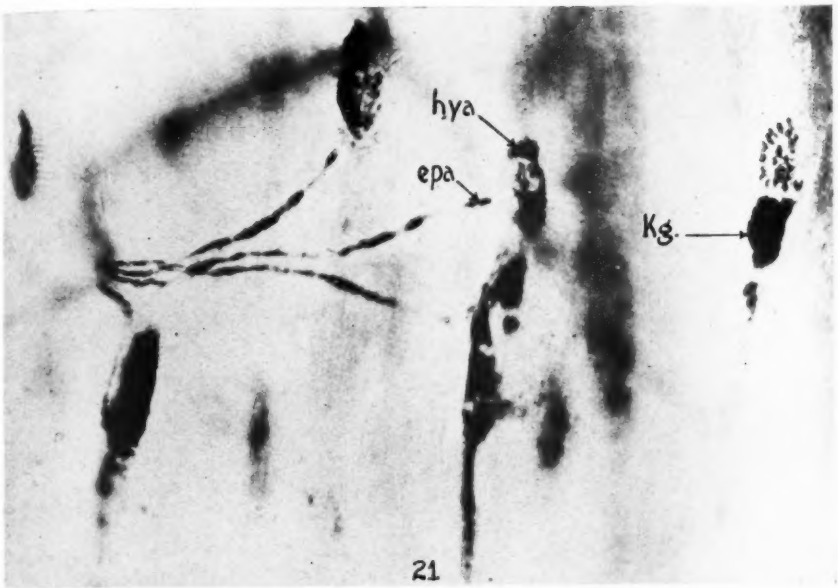
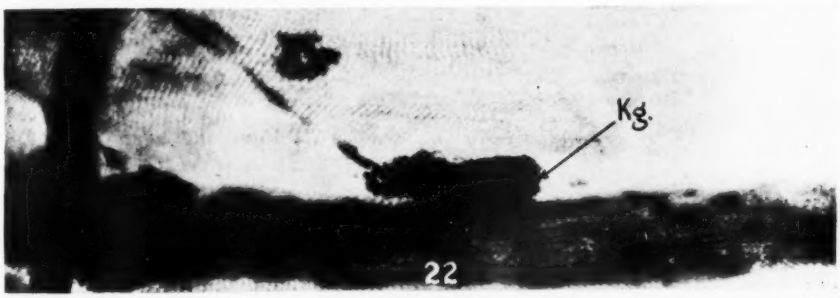
PLATE 164

FIGS. 21, 22, and 23. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes after subjection to trauma. There is an irregular dilatation and constriction of the epilemmal axons (epa). The hypolemmal axons (hya) are either retracted or expanded. The irregular accumulation and arrangement of the projected axonic material (Kg.) are clearly evident. These axonic droplets secreted by the motor end-plates take an intensive impregnation with gold and have either a unipolar (Fig. 22) or a typical bipolar arrangement (Fig. 23), or are irregular in arrangement and form elongated streamers (Fig. 21). These elongated streamers may or may not be cross-striated. In some places the cross striations of the axonic streamers conform with the periodicity of those of the muscle fibers. In other locations there is no agreement in the periodicities of these variable striations. × 350.





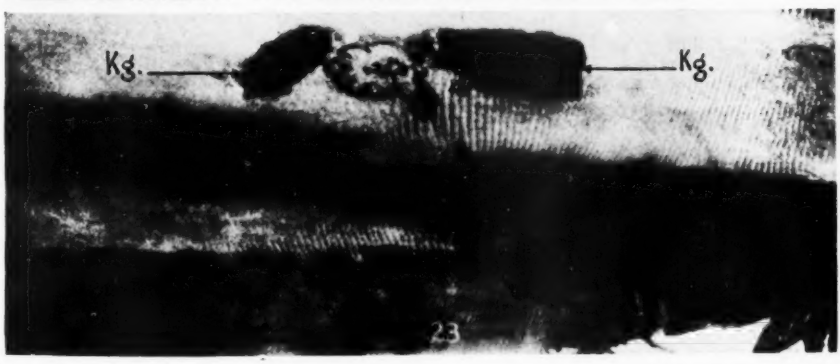


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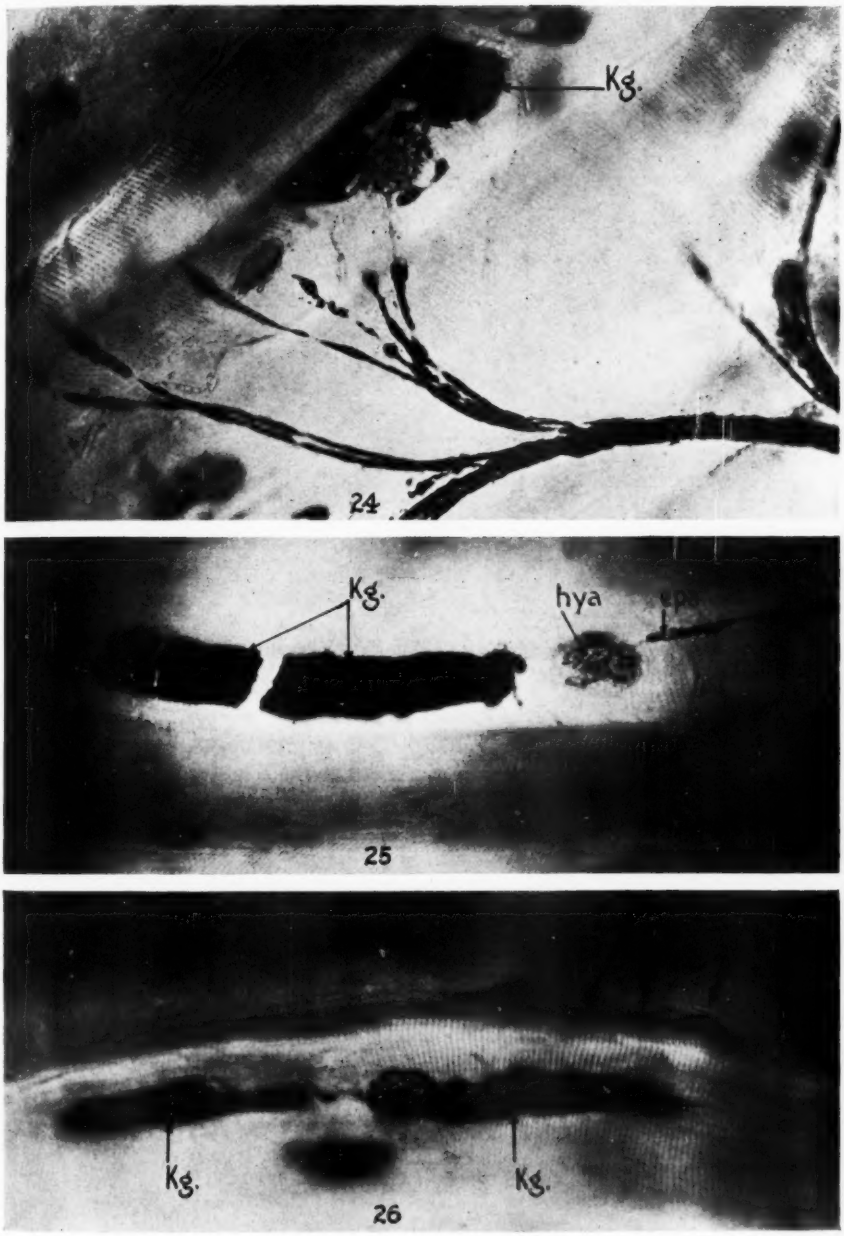
Plate 165

Figs. 24, 25, and 26. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. There is a semicircular arrangement of the dark mass of Kühne's granules (Fig. 24, Kg.) about the motor end-plate. An irregular arrangement of Kühne's granules is present at the pole opposite the one in which there is a massive accumulation of these granules. To the left of the motor end-plate there is a unipolar arrangement of the large masses of Kühne's granules (Fig. 25, Kg.). These masses that take an intense impregnation with gold represent an accumulation of axonic material that drains out of the motor end-plate. There is a bipolar arrangement of the large masses of Kühne's granules to the left and right of the motor end-plate (Fig. 26, Kg.), and a large dark droplet of axonic material in the center of this motor end-plate. × 350.



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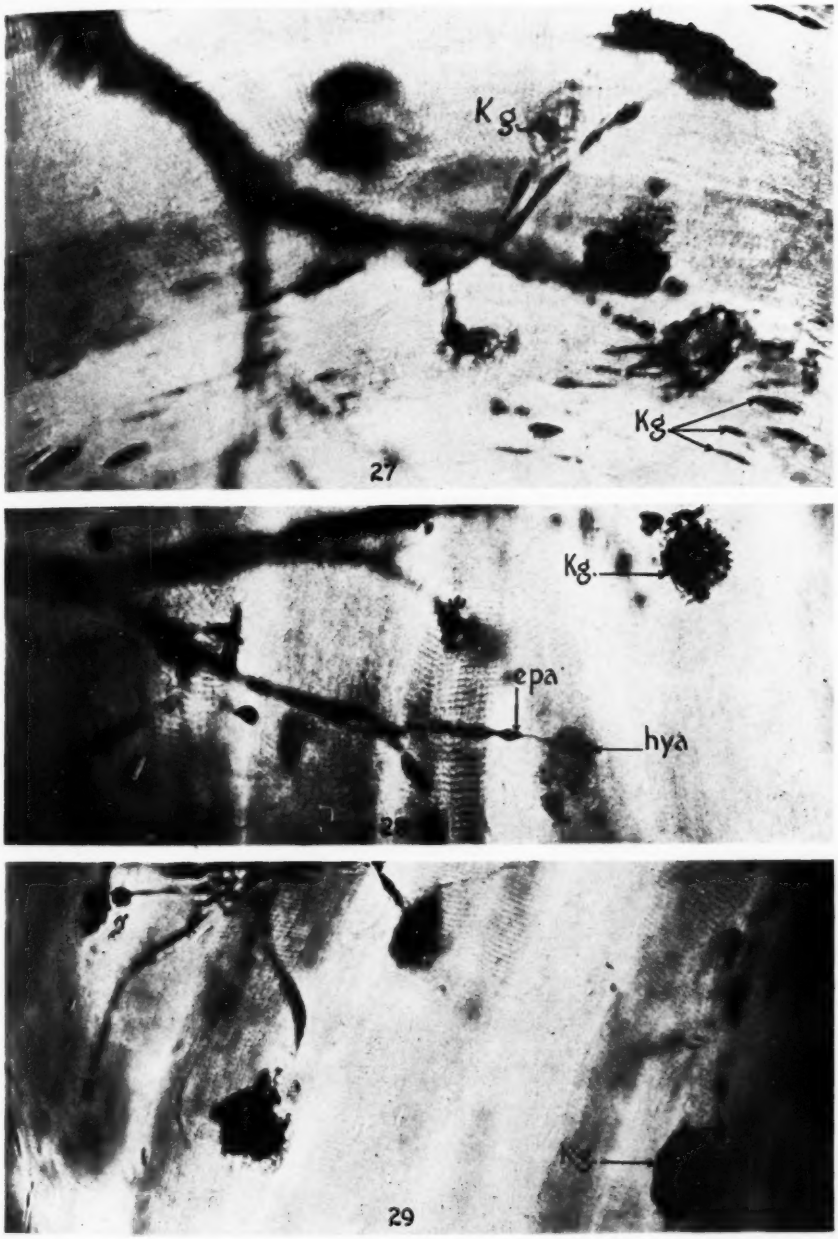
Neuromuscular Apparatus and Traumatic Shock

Plate 166

Figs. 27, 28, and 29. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya), and dark-staining masses of Kühne's granules (Kg.) found at variable distances from the motor end-plates in the muscle fibers. The dark-staining axonic material is found in the center of the end-plate, in a unipolar position external to the end-plate, as well as in a bipolar arrangement (Fig. 27). Small fusiform masses of axonic material (Kg.) are found projected from the lower motor end-plate (Fig. 27), and extend into the muscle fiber a considerable distance away from the end-plate. Some of these dark-staining fusiform masses are cross-striated. Others have a serrated appearance around the periphery. Concomitant with the projection of these axonic masses in the muscle fiber, there is in some locations a depletion of the epilemmal and hypolemmal axons. There is a central appearance of axonic material in the motor end-plate and a unipolar arrangement of this material (Fig. 28, Kg.). There is a large ovoid droplet of axonic material related to the motor end-plate below and to the right of the motor tree (Fig. 29, Kg.). These dark-staining masses of Kühne's granules (Kg.) appear to represent an abnormal secretion of nervous liquid into the muscle fiber. The abnormal appearance of this nervous material is evanescent and the material is subsequently dissolved; therefore large amounts of muscle material must be teased in order to detect the abnormal appearance of this nerve material in muscle. × 350.



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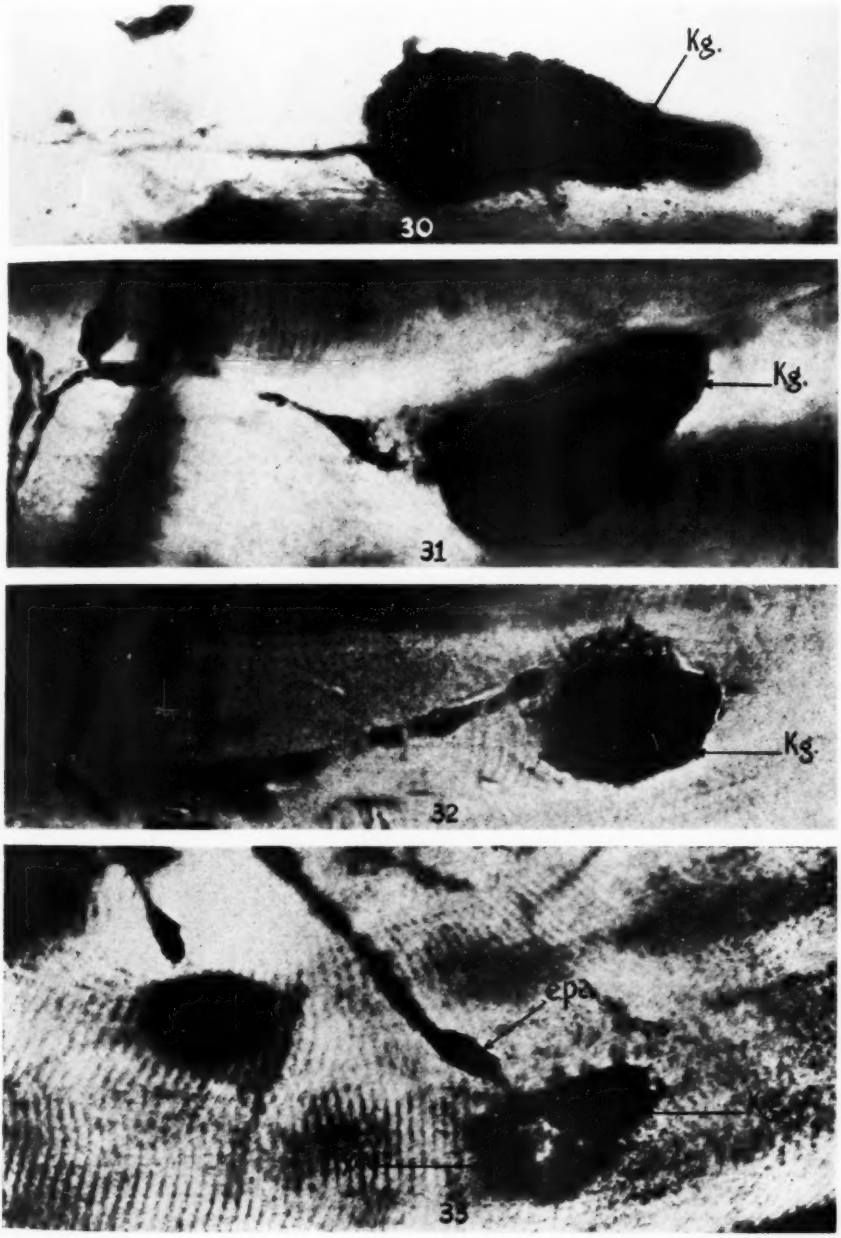
Neuromuscular Apparatus and Traumatic Shock

PLATE 167

FIGS. 30 to 33. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 15 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya), and dark-staining masses of Kühne's granules (Kg.). There is an oblong mass of Kühne's granules which overlies the motor end-plate (Fig. 30, Kg.). A dark ovoid mass of Kühne's granules is found in a unipolar position to the motor end-plate opposite to the pole where the union of the epilemmal axon is found (Fig. 31, Kg.). There is a flattened spherical mass of Kühne's granules at one pole of the motor end-plates (Figs. 32 and 33). × 750.



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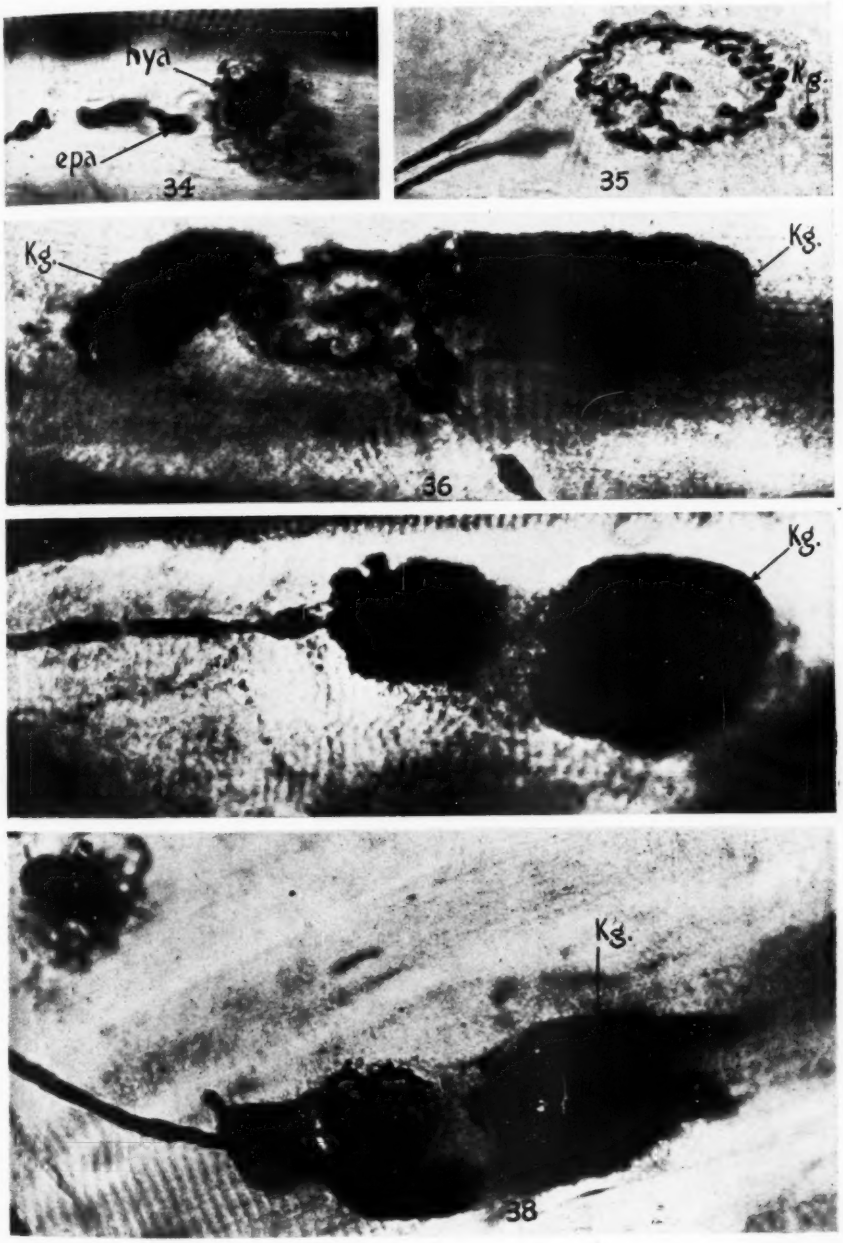
Neuromuscular Apparatus and Traumatic Shock

PLATE 168

FIGS. 34 to 38. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 30 minutes subsequent to traumatization. Different stages are found in the transmission of axonic material through the beaded epilemmal axons (epa), hypolemmal axons (hya), and dark-staining masses of Kühne's granules (Kg.). There is a large, dark droplet of Kühne's granules in the center of the motor end-plate (Fig. 34, Kg.), and a small spherical droplet of Kühne's granules external to the motor end-plate and at the pole opposite to that of the continuity with the epilemmal axons (Fig. 35, Kg.). A bipolar arrangement of large oblong masses of Kühne's granules is extrinsic to the hypolemmal axons of the end-plate (Fig. 36, Kg.). There is a unipolar arrangement of the large, dark, discoidal mass of Kühne's granules at the pole opposite to that which is continuous with the epilemmal axon (Fig. 37, Kg.). An irregular arrangement of the large, dark masses of Kühne's granules is shown about the motor end-plate (Fig. 38, Kg.). × 750.



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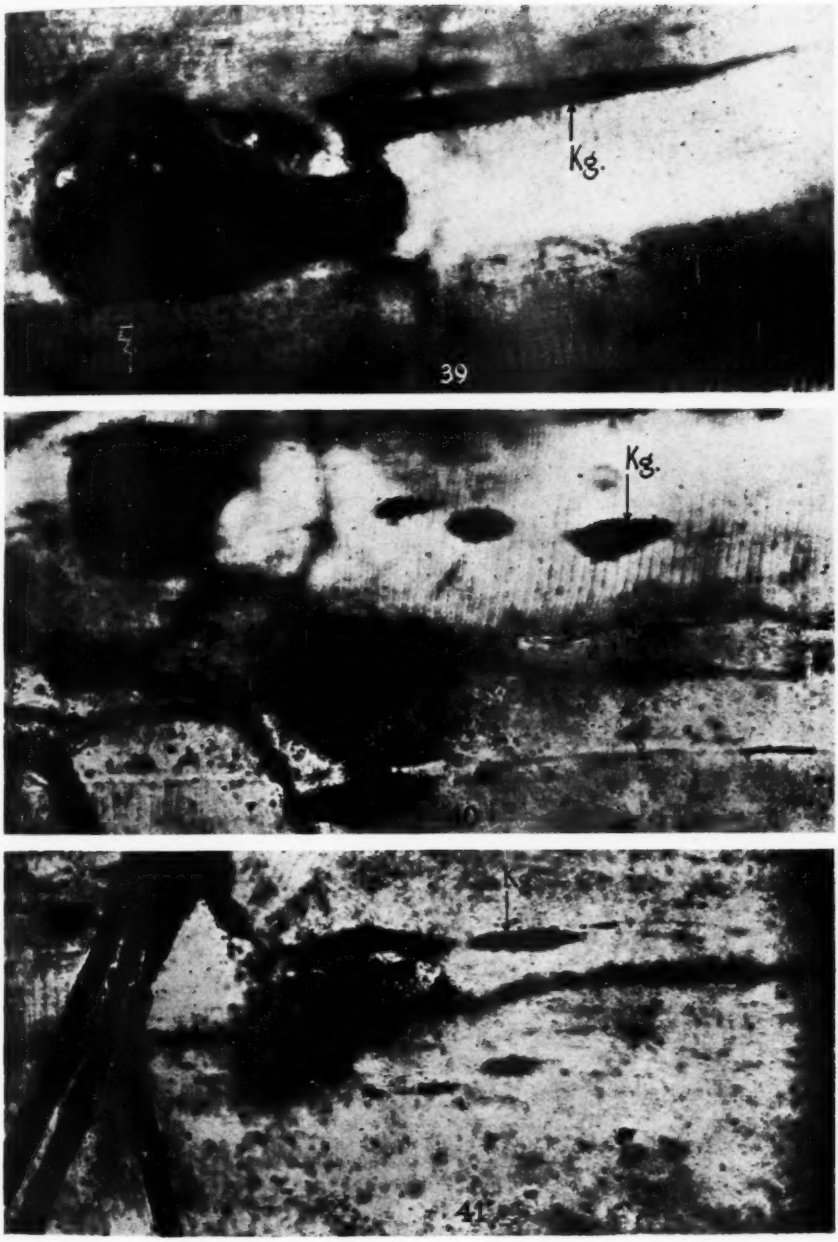
Neuromuscular Apparatus and Traumatic Shock

PLATE 169

FIGS. 39. 40. and 41. Motor end-plates from gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. An elongated streamer of Kühne's granules (Kg.) is observed to the right (Fig. 39) of the motor end-plate. Discrete and fusiform masses of these granules are projected to the right (Figs. 40 and 41) of each motor end-plate. In some places these masses of granules are homogeneous, and in others they have a cross-striated structure. × 750.



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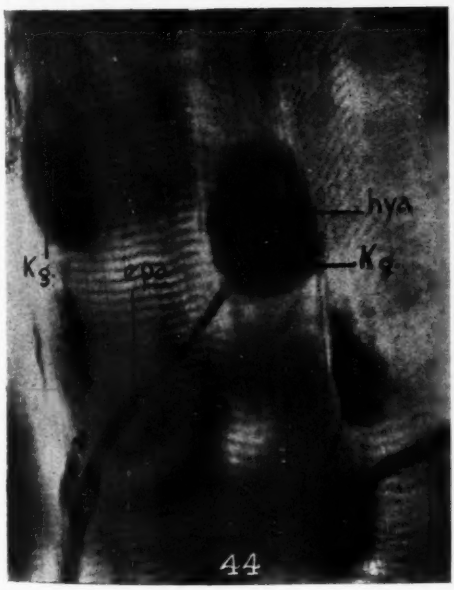
Neuromuscular Apparatus and Traumatic Shock

PLATE 170

FIGS. 42 to 45. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. An elongated streamer of Kühne's granules (Fig. 43, Kg.) is observed extending downward from the dark, retracted end-plate. Some end-plates are in a state of abnormal retraction (Fig. 42) and take an intense impregnation with gold. Other end-plates are relatively normal and have a clear, halo-like space between the hypolemmal axons and the rim of Kühne's granules (Fig. 44). Abnormal retraction and hyperchromatophilia for gold are the first effects of the local injection of lactic acid experimentally into muscle. This first phase of retraction and hyperchromatophilia is quickly followed by the second phase of expansion, projection of axonic material into the muscle, disintegration, and complete liquefaction. × 750.



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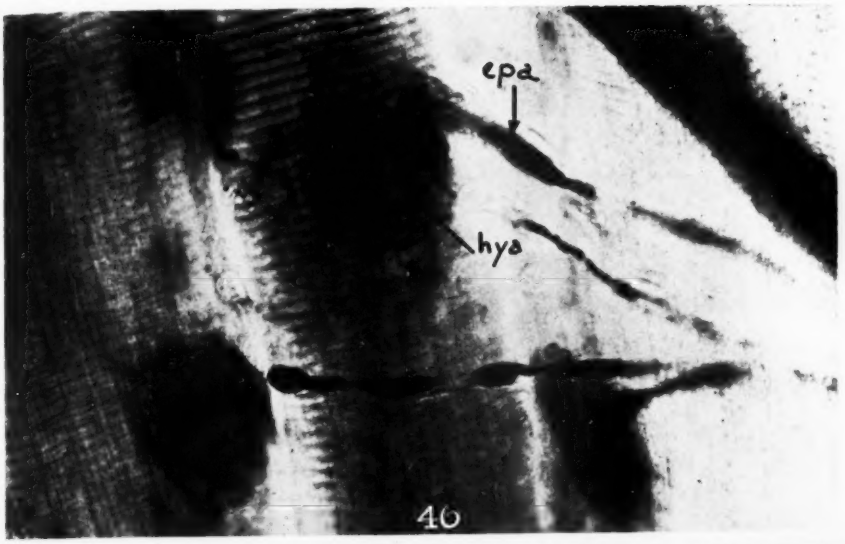
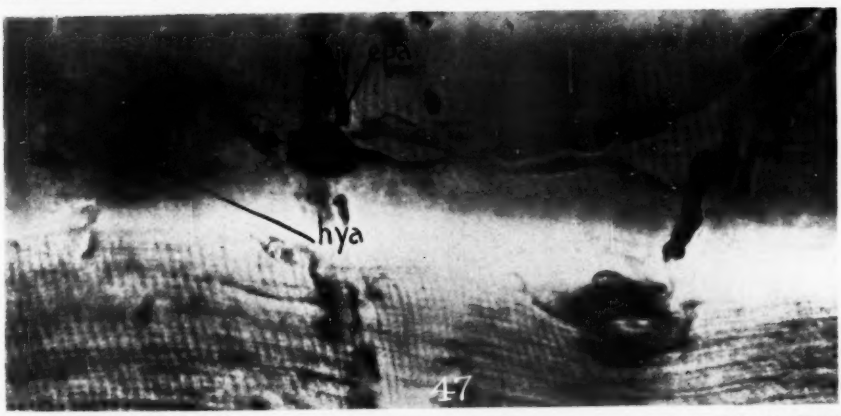
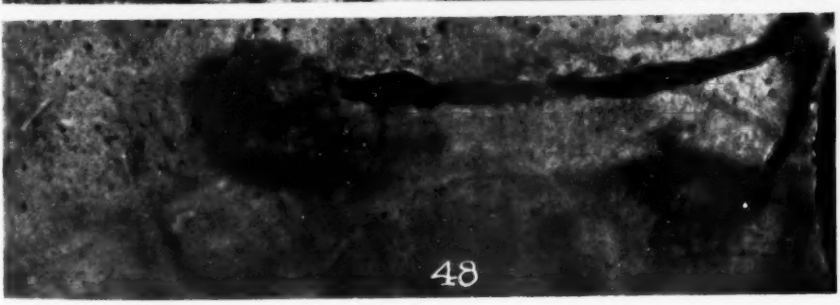
Neuromuscular Apparatus and Traumatic Shock

PLATE 171

FIGS. 46, 47, and 48. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons (epa). Some of the hypolemmal axons (hya) of the motor end-plates are relatively normal and have variable degrees of expansion and quantitative condensation of Kühne's granules (Fig. 46). In some places there is a diminished affinity of the end-plate for gold with a corresponding granular degeneration of both the motor end-plates and muscle fibers (Fig. 48). One end-plate to the left has a central island of Kühne's granules (Fig. 48). × 750.







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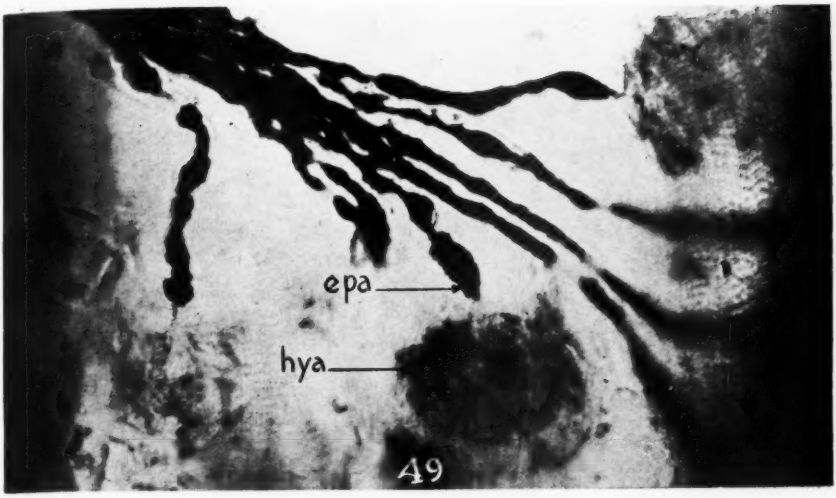
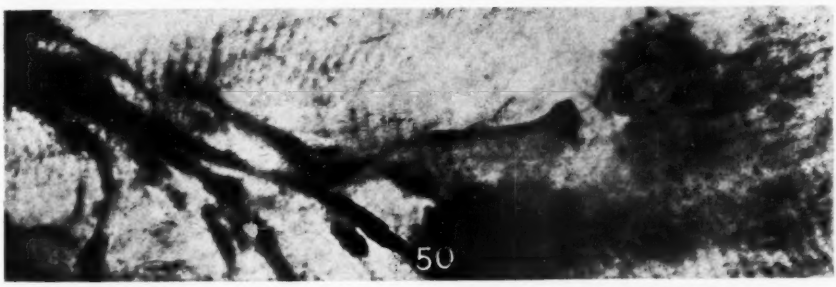
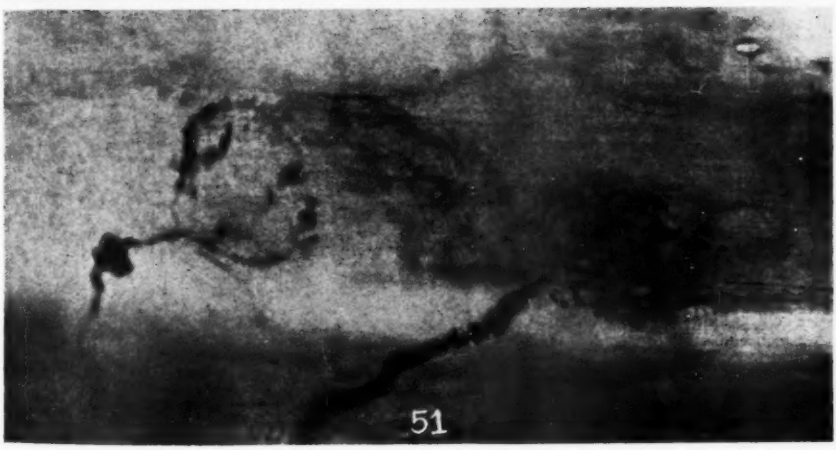
Neuromuscular Apparatus and Traumatic Shock

PLATE 172

FIGS. 49, 50, and 51. Motor end-plates from gastrocnemius muscle of an unanesthetized rat 15 minutes after traumatization. There is an irregular moniliform pattern of the epilemmal axons (epa). The hypolemmal axons (hya) of the motor end-plates have variable degrees of expansion and liquefaction. In some of the motor end-plates there is practically complete disappearance of the hypolemmal axons and of the granules of Kühne. These morphologic changes in the motor end-plates produced by traumatic shock are comparable to those caused by the injection experimentally of lactic acid directly into living, nerve-intact muscle. × 750.







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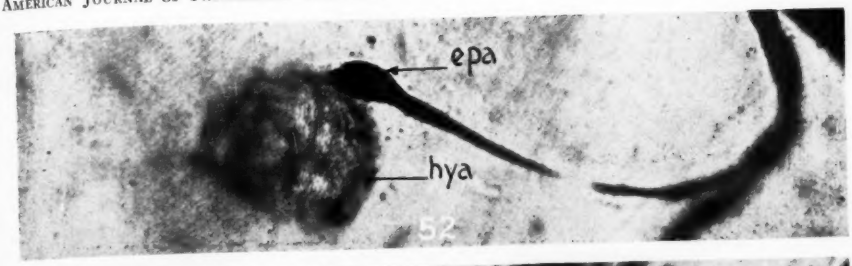
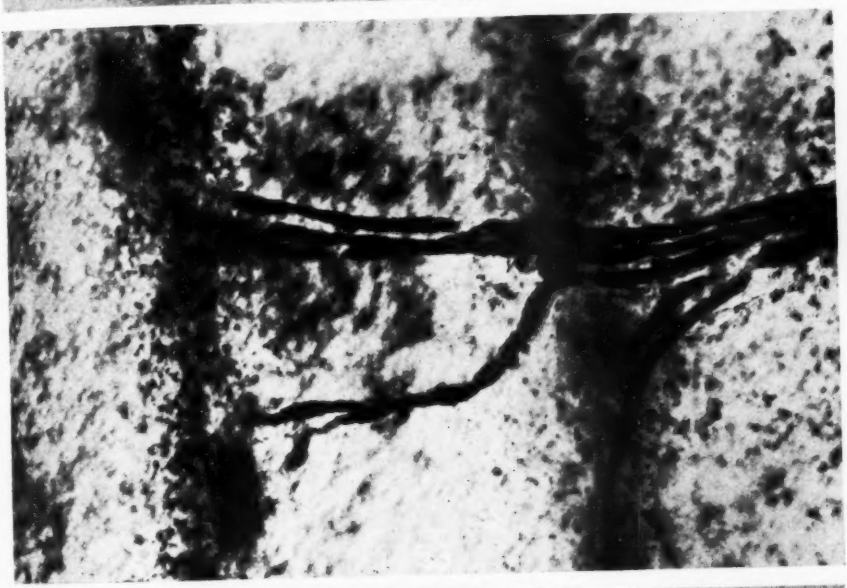
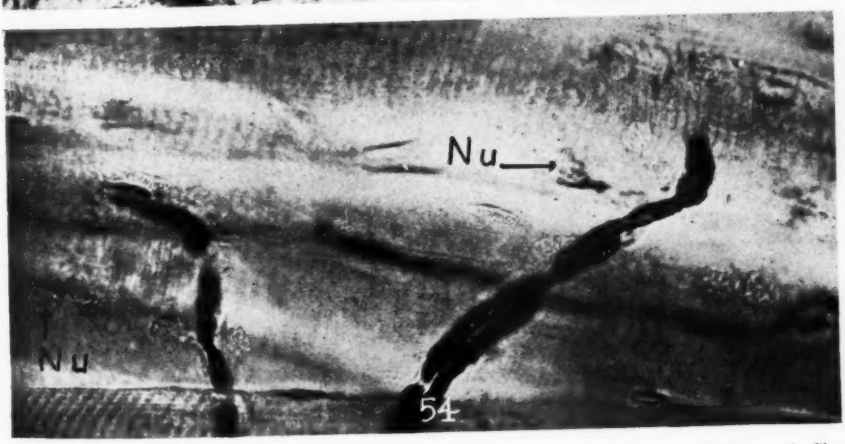
Neuromuscular Apparatus and Traumatic Shock

PLATE 173

FIGS. 52, 53, and 54. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons. An especially large bulb of the epilemmal axon (epa) is found at its junction with the hypolemmal axons (hya) (Fig. 52). These hypolemmal axons are undergoing liquefaction (Fig. 52). There is an irregular dispersal of granules in relation to some of the disintegrating motor end-plates (Fig. 53). In other locations of the same muscle the hypolemmal axons have completely disappeared. There is, likewise, a complete disappearance of the granules of Kühne (Fig. 54). The clear, rounded and oval areas in the location previously occupied by the hypolemmal axons are the outlines of the nuclei related to the motor end-plate in the muscle fiber (Fig. 54, Nu). × 750.







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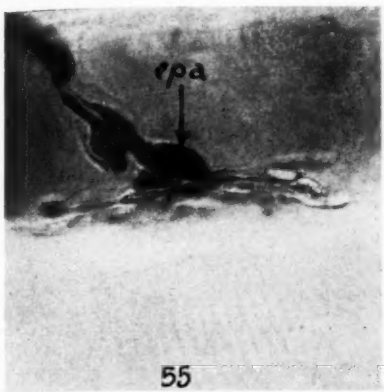
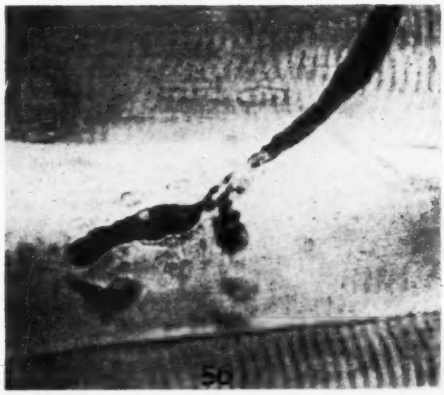
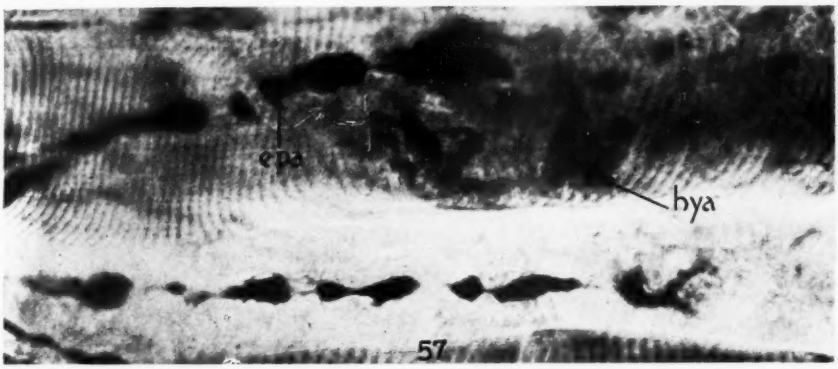
Neuromuscular Apparatus and Traumatic Shock

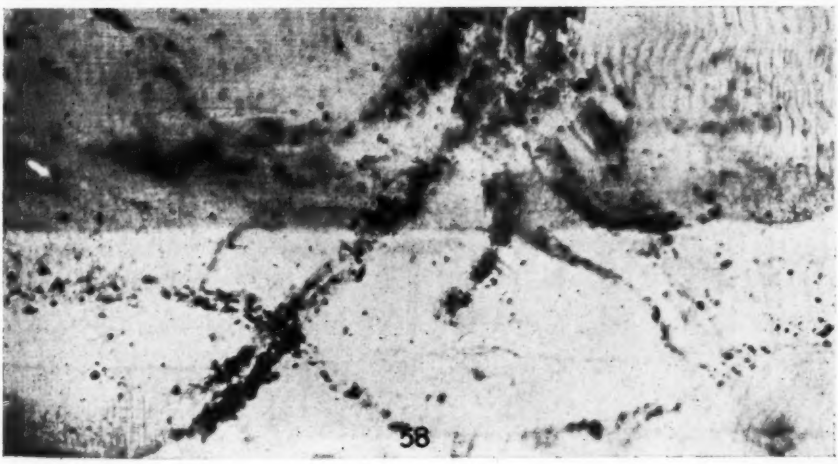
PLATE 174

FIGS. 55 to 58. Motor end-plates from the gastrocnemius muscle of an unanesthetized rat 15 minutes after subjection to trauma. There is an irregular moniliform pattern of the epilemmal axons (epa). There is a complete disappearance of the hypolemmal axons of the motor end-plates in some locations (Figs. 55, 56, and 58). Remnants of the hypolemmal axons (hya) and granules of Kühne are found in other locations (Fig. 57). In certain areas the epilemmal axons are undergoing a complete granular degeneration (Fig. 58). × 750.









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Neuromuscular Apparatus and Traumatic Shock

PLATE 175

FIGS. 59, 60, and 61. Gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. Sprays of medullated nerve fibers (Fig. 59) are denuded of motor end-plates. Some of the muscle nuclei exhibit increased visibility and there are irregular coagulation bands of muscle tissue, some of which are opaque and others finely cross-striated. In some locations the blood vessels are dilated and packed with red blood cells (Fig. 60), with incipient perivascular infiltration of polymorphonuclear leukocytes. The morphologic lesion of the neuromuscular apparatus produced by trauma is a structural expression of the profound alteration in the underlying biochemical processes of nerve and muscle. In still other locations the cross striations of the muscle fibers are replaced by a dense granulation that forms oblong or fusiform structures (Fig. 61). These acute degenerative changes are comparable to those produced by the local injection of lactic acid into the muscle. × 125.

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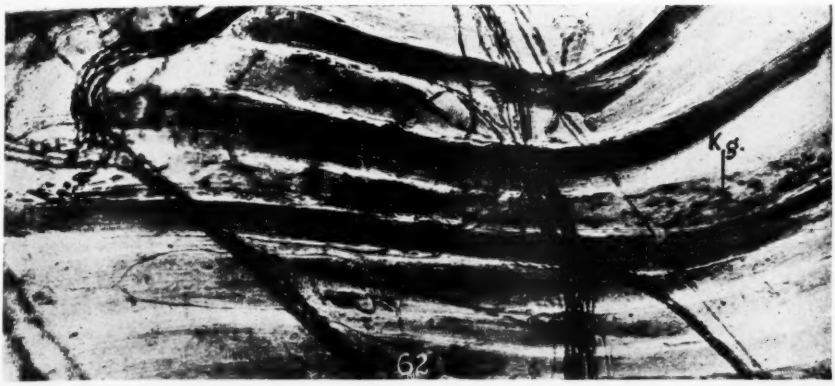
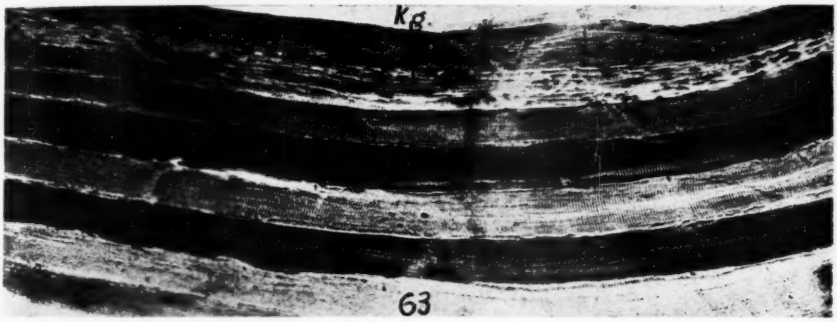
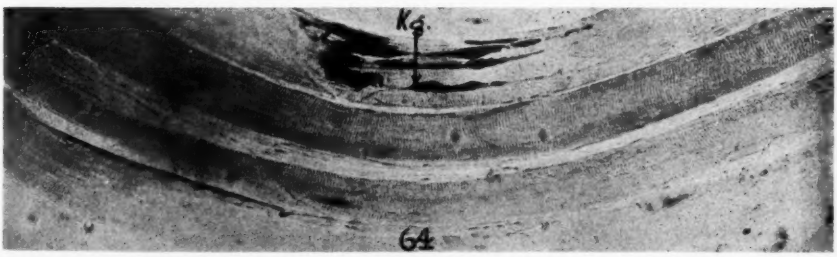
Neuromuscular Apparatus and Traumatic Shock

Plate 176

Figs. 62 to 65. Gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. Sprays of medullated nerve fibers are found, some of which are denuded of their motor end-plates. In one of the fibers (Fig. 62) there are oblong and fusiform gold-impregnated masses scattered throughout the myoplasm. This material appears in the muscle fiber coincident with the disappearance of the end-plate and the depletion of axonic materials from the epilemmal axons. Some of the muscle fibers retain their cross-striated structure and others show an intermingling of the cross striations and the gold-impregnated axonic substance (Figs. 62 to 65, Kg.). This axonic substance may or may not be cross-striated. If striated, it may or may not have the periodicity of its bandings coincide with that of the cross striations of the muscle. In certain locations this axonic material may form greatly elongated and fragmented streamers between the muscle fibers. These may be 500 to 1000 μ in length (Figs. 64 and 65, Kg.). This axonic material (Kg.) may be found either within or without the sarcolemma of the muscle fiber. $\times$ 125.







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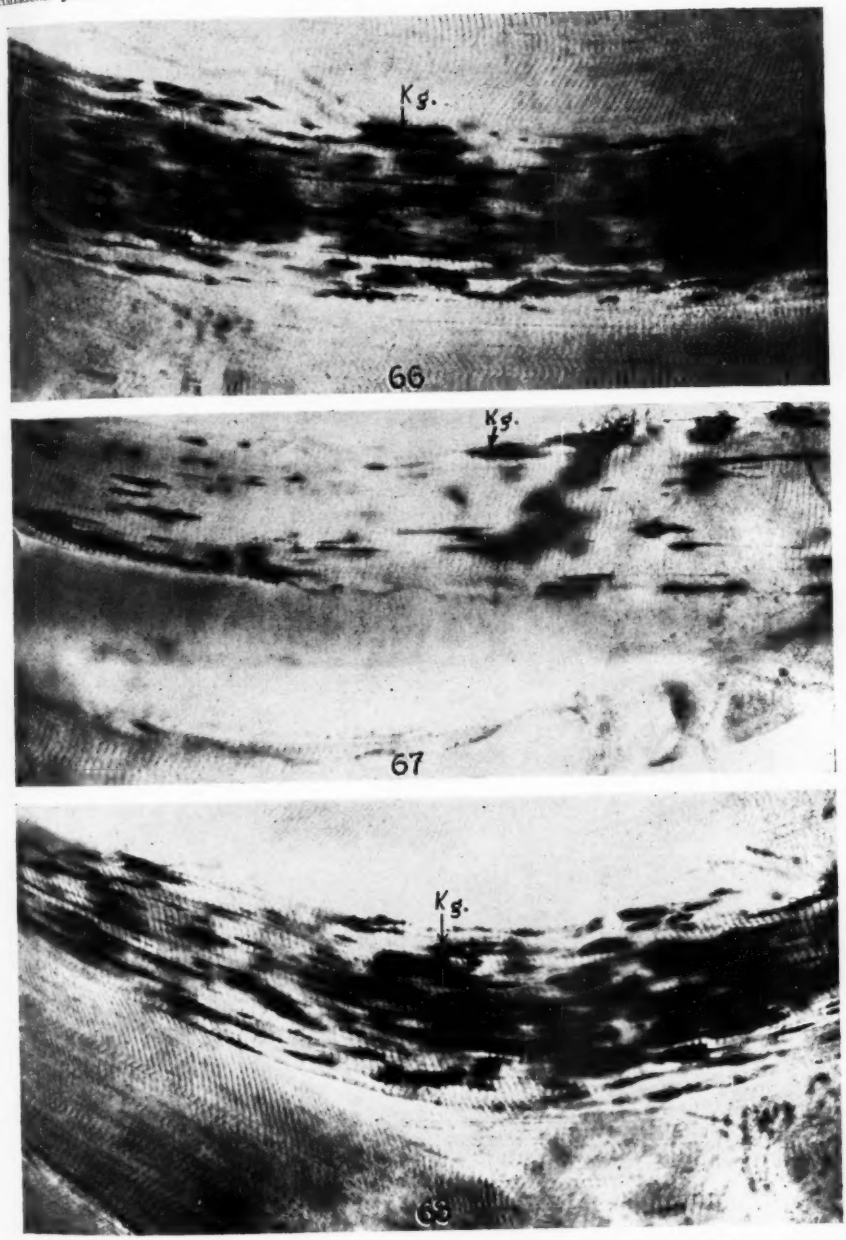
Neuromuscular Apparatus and Traumatic Shock

PLATE 177

FIGS. 66, 67, and 68. Gastrocnemius muscle fibers from an unanesthetized rat 15 minutes after subjection to trauma. In some muscle fibers the discharged axonic material (Kg.) that is hyperchromatic for gold is densely crowded into oblong and fusiform masses. In many fibers of the same muscle these gold-impregnated masses are absent. This projection of auriphilous material was not found in the bilateral control muscles from ten rats. These masses are either opaque with serrated borders or cross-striated. Their cross striations may or may not conform with those of the muscle fiber in which they are found. These ephemeral masses of projected axonic material are found only during the time of, and shortly after, muscle trauma. Within 1 to 2 hours after traumatization they disappear. Their appearance in muscle is coincident with that of morphologic depletion of the motor innervation of its axonic material; and their disappearance, with the beginning of granular degeneration of some of the muscle fibers. The local injection of lactic acid into muscle produces a similar structural depletion of motor innervation and the ephemeral appearance of axonic masses in the muscle. The increased acidity, experimentally produced locally in muscle, augments the permeability of the end-plates and structurally exhausts the axonic conduction medium of the innervation of muscle. × 750.



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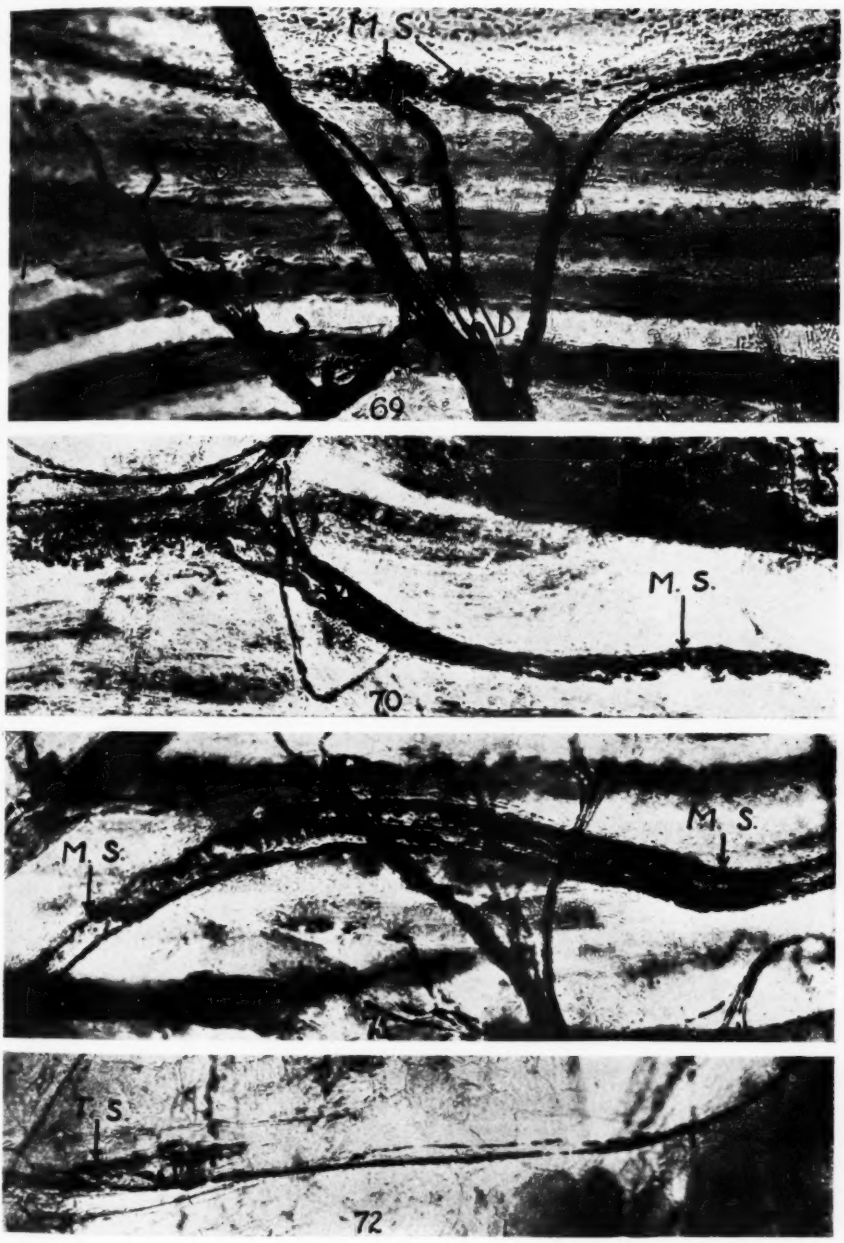
Neuromuscular Apparatus and Traumatic Shock

PLATE 178

FIGS. 69 to 72. Proprioceptive muscle spindles (Figs. 69 to 71, M.S.) in the gastrocnemius muscle from an unanesthetized rat 15 minutes after subjection to trauma. In some places the sensory and motor terminals of the spindle are fragmented. The afferent sensory axons of the spindle are enlarged and in some places (Fig. 69, D) greatly dilated and hyperchromatic for gold. The epilemmal axons (epa) are denuded of their hypolemmal axons that form the motor end-plates. The increased visibility of the dark nuclei (Fig. 69) of the muscle fibers is comparable to that produced by the experimental injection of lactic acid locally into the muscle. The altered structure of motor and sensory nerve endings and of the muscle fibers and blood capillaries produced by trauma appears to parallel the alteration of metabolism resulting from anoxia produced by the experimental injection of lactic acid locally into muscle. The increased concentration of lactic acid increases the permeability of the end-plate which results in an augmented outflow of the axonic substance into the muscle. The tendon spindle (Fig. 72, T.S.) is composed of branched terminals that end in club-shaped enlargements. There is some evidence of axonic fragmentation. × 125.



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Neuromuscular Apparatus and Traumatic Shock

UNIV. OF MICH.

CELLULAR REACTIONS TO MYCOLIC ACIDS*

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Mycobacteria may be differentiated from other microorganisms in their content and variety of fatty acids, phosphatides and wax-like substances. The latter, when isolated chemically,[1] are acid-fast and are assumed to be responsible for the staining characteristics of the mycobacteria.[2] These waxy substances, particularly those of the tubercle bacillus, were considered as a waxy shell [3] but this hypothesis has been abandoned.[4] Sabin, Smithburn, and Thomas,[5] working with rabbits, reported that these waxes neither produced necrosis nor damaged phagocytes in which they were contained. Thomas and Dessau,[6] on the other hand, recorded granulomata, including necrosis and giant cell formation, quite similar to the lesions resulting from living tubercle bacilli, following inoculation of mice. The waxes used by both groups of workers [5, 6] were unpurified, and derived from human and bovine tubercle bacilli as well as from leprosy bacilli.

Thomas [7] later reported production of granulomata containing many multinucleated giant cells by intraperitoneal administration in rabbits of phthiocerol, a higher alcohol derived from human tubercle bacilli. These bore no other resemblance to the granulomata of tuberculosis.

The materials used in the studies referred to were products of the extensive investigations of Anderson and his associates. More recently these investigators have isolated hydroxy acids by high temperature fractionation of the wax from various acid-fast organisms.[8] Through the courtesy of Dr. Anderson, it has been possible to study the reaction to several of these long chain fatty acids identified as mycolic acids. They include low and high melting point varieties derived from human and also from bovine and avian strains of tubercle bacilli as well as from leprosy bacilli (Table I).

TABLE I

1. Mycolic acid derived from human strain of tubercle bacillus	M.P. 55–56°
2. Mycolic acid derived from human strain of tubercle bacillus	M.P. 73–75°
3. Mycolic acid derived from leprosy bacillus	M.P. 62–63°
4. Mycolic acid derived from bovine tubercle bacillus	M.P. 56–58°
5. Mycolic acid derived from avian tubercle bacillus	M.P. 60–61°

* These studies have been aided by funds provided by the Research Committee of the National Tuberculosis Association, and are a part of a plan of cooperative research sponsored by the Research Committee.

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At 37°C. these mycolic acids are white amorphous powders that tend to clump so that homogeneous suspensions for animal inoculation are difficult to prepare, as has been noted previously with other waxes by Sabin, Smithburn, and Thomas [5] and by Thomas and Dessau.[6] By chilling and grinding in a homogenizer similar to that recommended by Corper and Cohn [9] and suspending as suggested by Anderson in 6 per cent gum arabic, a satisfactory preparation for prompt injection is secured. For intracutaneous injection 0.25 cc. of such suspension, containing 5 mg. of acid, was utilized and 4 cc., containing 20 mg. of acid, for intraperitoneal injection. For intravenous injection, 16 mg. of mycolic acid plus 2 to 3 cc. of distilled water were homogenized and further diluted with distilled water to which monoethanolamine (Eastman) had been added. The pH was then adjusted to 8.3 with normal hydrochloric acid and the volume adjusted so that each cc. contained 1 mg. of acid. At 37°C. this suspension became milky and contained particles of varying size as determined microscopically. With low-melting acids from human or avian tubercle bacilli, the particles were the size of a red blood cell; in the other preparations particles as large as polymorphonuclear leukocytes were seen. As a rule, 4 cc. of such a suspension was injected intravenously at intervals of 4 days until four treatments had been given. Several rabbits received an injection of 16 mg. for the study of changes during the first to fifth days.

Results

A. Intracutaneous Injection of Mycolic Acids

Gross observation, including measurement and classification of the lesions according to the degree of inflammation that resulted from intracutaneous injection, was carried out on five rabbits over a period of 45 days. Each of these animals was injected intracutaneously with three of the acids. Lesions induced by each of the acids could, therefore, be observed in three different rabbits.

The lesions resulting from the high-melting human mycolic acid and leprosinic acid were small and little evidence of inflammation was seen. They started to subside in all three rabbits about the 15th day. In contrast, the lesions following the injection of low-melting human mycolic acid and bovine mycolic acid became large and widespread on the 15th to 22nd day and in two of the rabbits the lesions were still distinct on the 39th day after injection. An initial inflammatory response followed the injection of avian mycolic acid. The lesions, however, were small on the 15th day and started to subside about the 22nd day. The point of injection of gum arabic alone as a control could not be identified 5 days after administration.

In a second series, lesions resulting from the intracutaneous injection of mycolic acid were excised on the 5th, 10th, 15th, 25th, and 40th to 45th day and studied microscopically. An initial stage of necrosis and of polymorphonuclear reaction was observed in every instance. Later the lesions were divisible into two groups. The injection of bovine mycolic acid and low-melting human mycolic acid was followed by formation of giant cells by the 5th day. These cells became large and their cytoplasm foamy by the 10th day. Deposit of calcium in both the lesions of low-melting human and bovine mycolic acid, and necrosis in the latter, 45 days after injection, furnished an additional differentiating factor.

In the lesions produced with the other three acids, giant cells were seen only on the 15th day. Slit-like cytoplasmic defects were present in them, apparently corresponding to the ingested mycolic acid. The fibrous tissue proliferation was moderate. Necrosis was absent in sections obtained after the 15th day and no calcium deposit was found.

B. 1. Intraperitoneal Injection of Mycolic Acids in Rabbits

Twenty-two rabbits were included in the group. Each received a single intraperitoneal injection of 20 mg. of acid suspended in gum arabic.

On the 15th day after injection, the low-melting human mycolic acid and the bovine mycolic acid distinguished themselves by producing large granulomatous lesions and extensive cellular disintegration (Figs. 1 and 3). The former were composed of multinucleated giant cells, numerous monocytes, and small round cells as well as polymorphonuclear leukocytes. Moderate to marked fibroblastic proliferation was noted. The giant cells were large, their nuclei numerous and the cytoplasm had assumed a foamy appearance due to the presence of many small vacuoles. At the 45th day, the lesions were still similar and showed the same cellular disintegration; numerous foamy monocytes and deposition of calcium were noted (Figs. 5 and 6).

Giant cells arranged in small groups, some monocytes and polymorphonuclear leukocytes characterized the lesions induced by high-melting human mycolic acid, leprosinic acid, and avian mycolic acid at the 15th day. Only a small amount of cellular disintegration was noted in the lesions resulting from the injection of leprosinic acid. The response to avian mycolic acid was minimal. Gum arabic itself induced giant cell formation of a slight degree. The giant cells contained a few nuclei and no vacuoles. This cellular reaction disappeared after the 15th day (Figs. 2 and 4).

On the 45th day the omenta of the rabbits injected with high-melting human mycolic acid were free of lesions save for a small amount of

fibrous tissue. The omenta of the rabbits that had received the avian mycolic acid revealed, in addition, an occasional group of two to four giant cells. The cellular disintegration found in the earlier lesions produced by leprosinic acid had disappeared, although the presence of the injected material within the giant cells could be demonstrated. The giant cells in this group were smaller, the nuclei less numerous, than in the first group, while the cytoplasmic defects were large and replaced much of the cell protoplasm.

The omental preparations obtained from this group of animals were of particular value in demonstrating that the vacuoles of the giant cells, as noted in sections secured by the usual technic, corresponded to the phagocytosed mycolic acid. Its identification was facilitated by acid fastness. Paraffin imbedding and its requisite fat solvents removed the mycolic acids. The frozen section method or imbedding in polyvinyl alcohols did not furnish sections of sufficient quality.

B. 2. Intraperitoneal Injection of Mycolic Acids in Guinea-Pigs

The structure of the lesions that resulted from intraperitoneal injection of the various mycolic acids in 20 guinea-pigs did not reveal differences as characteristic as those observed in rabbits. Granulomatous lesions composed of numerous multinucleated giant cells and vacuolated monocytes with a few fibroblasts interspersed were always present. The appearance of the giant cells did not vary with the different acids. Two weeks after injection, necrosis was found in all lesions except those induced with high-melting human mycolic acid.

Microscopic preparations obtained 6 weeks after the administration of the acids showed granulomatous lesions made up of numerous, round, multinucleated giant cells and some monocytes. The cytoplasmic defects in the case of the low-melting human mycolic acid were less numerous and smaller. A few fibroblasts were present between the giant cells. There was no evidence of cellular disintegration. The only lesion with well developed fibrous connective tissue resulted from avian mycolic acid.

C. Intravenous Injection of Mycolic Acid in Rabbits

This group comprised 33 animals. Each mycolic acid was injected as a suspension in water-ethanolamine. The latter compound alone produced no lesion.

A single intravenous injection of any of the mycolic acids caused, initially, intensive damage to pulmonary vessels as indicated by fibrinoid necrosis of the vessel walls and polymorphonuclear exudation throughout the vessel coat. No differentiating morphologic character-

istics for the various acids were found. Occasional giant cells with vacuolated cytoplasm were seen in the lumina of small arteries of the lung. Slight differences, depending upon the acid used, were found in the pulmonary lesions of rabbits sacrificed 24 hours after the last of four intravenous injections. The lesions of the low-melting human mycolic acid and of the bovine mycolic acid were more numerous, and consisted of polymorphonuclear leukocytes and monocytes surrounding giant cells that contained injected acid. The difference became more apparent on the 5th day, when the lesions induced by low-melting human mycolic and bovine mycolic acid were conspicuous both by their frequency and their granulomatous character (Fig. 7). Marked polymorphonuclear reaction was still present. The latter type of response was only slight with the other acids and absent with the high-melting human mycolic acid.

The giant cells with their contained low-melting human or bovine mycolic acid displayed several small vacuoles; those developed from the other acids exhibited large cytoplasmic defects (Figs. 8 and 9). The giant cells resulting from the injection of low-melting human or bovine mycolic acid (Fig. 7) contained many nuclei, those from the other acids fewer nuclei (Figs 8 and 9). These lesions were found exclusively in the lung.

At 15 and 45 days after injection, the frequency of the lesions in rabbits injected with low-melting human or bovine mycolic acid contrasted sharply with the rare ones after high-melting human mycolic, leprosinic or avian mycolic acid. The lesions of the two groups retained their different character. After low-melting human or bovine mycolic acid they were granuloma-like, containing large giant cells with numerous nuclei and vacuolated monocytes (Figs. 10 and 14). After the other three there were only isolated giant cells without further cellular reaction (Figs. 11, 12, 13, and 15).

At 45 days, small groups of vacuolated giant cells were found in the spleens, and occasionally in the livers, of animals injected with any of the mycolic acids.

Discussion

The mycolic acids, products obtained by fractionation of the waxes of tubercle bacilli and other acid-fast microorganisms, are largely responsible for the characteristic physicochemical properties of the waxes, including acid fastness. Lesions induced by them, therefore, might be expected to simulate those resulting from the waxes as described by Sabin, Smithburn, and Thomas.[5] This, in general, is the case. The initial polymorphonuclear reaction and secondary accumulation of

monocytes and of multinucleated giant cells, as observed in these investigations, correspond to lesions reported by Sabin *et al.*

The cellular response to the mycolic acids differs from that of the waxes inasmuch as all of the former result in initial and marked necrosis. This persists with low-melting human, and particularly with bovine, mycolic acid.

Although mycolic acids are distinguishable chemically only with difficulty, they differ in the reactions they excite in the rabbit. By these reactions these acids are roughly divisible into two groups. Those of the first group, including high-melting human and avian mycolic and leprosinic acid, are only slowly fragmented into small particles and cause initial but nonpersistent necrosis. The subsequent lesions are small, consisting largely of giant cells that contain few vacuoles and are surrounded by a minimal proliferative reaction.

Acids of the second group, low-melting human and bovine mycolic acid, rapidly resolve into finer particles and bring about extensive and persistent necrosis. The lesions retain their character for several weeks.

No known chemical difference among various types of tubercle bacilli has shed any light so far upon the problem of native resistance. Variation in cellular response to the mycolic acids may be related to differences in susceptibility of animals to the strain of tubercle bacilli from which the acid is derived. This correlation is, however, incomplete. The rabbit is known to be susceptible to both bovine and avian tubercle bacilli. The mycolic acid recovered from the bovine strain is more effective in producing long-standing necrosis and extensive cellular response. Avian mycolic acid, however, in its biologic result, is not unlike that of the leprosy bacillus to which this animal is refractory. Reciprocally, low-melting mycolic acid from the human tubercle bacillus produces lesions similar to those caused by bovine mycolic acids although the rabbit is not susceptible to the latter mycobacterium.

The mycolic acids as constituents of the tubercle bacilli may be assumed, therefore, to contribute to the persistence of lesions induced by this microorganism, but there is no evidence that they are related to the elective pathogenicity of different types of tubercle bacilli.

REFERENCES

1. Anderson, R. J. The chemistry of the lipoids of tubercle bacilli. VIII and IX. *J. Biol. Chem.*, 1929–30, **85**, 339–354.
2. Anderson, R. J. The chemistry of the lipoids of tubercle bacilli. IV. Concerning the so-called tubercle bacilli wax. Analysis of the purified wax. *J. Biol. Chem.*, 1929, **83**, 505–522.
3. Sordelli, A., and Arena, A. Interprétation de l'acido-résistance du *Mycobacterium tuberculosis*. *Comp. rend. Soc. de biol.*, 1934, **117**, 63–64.
4. Lembke, A., and Ruska, H. Elektron mikroskopische Studien der Tuberkelbazillen. *Klin. Wchnschr.*, 1940, **19**, 19.

5. Sabin, F. R., Smithburn, K. C., and Thomas, R. M. Cellular reactions to wax-like materials from acid-fast bacteria. *J. Exper. Med.*, 1935, **62**, 751–769.
6. Thomas, R. M., and Dessau, F. I. Experimental tuberculosis in mice. The cellular response to the chemical fractions of the tubercle bacillus. *Yale J. Biol. & Med.*, 1939, **12**, 185–198.
7. Thomas, R. M. Cellular reactions to lipoid fractions of the tubercle bacillus. Phthiocerol, an alcohol derived from human and bovine tubercle bacilli. *Yale J. Biol. & Med.*, 1939, **12**, 283–286.
8. Anderson, R. J. The chemistry of the lipoids of the tubercle bacillus. *Yale J. Biol. & Med.*, 1943, **15**, 311–345.
9. Corper, H. J., and Cohn, M. L. A mechanical device for preparing fine suspensions of tubercle bacilli and other microorganisms. *J. Lab. & Clin. Med.*, 1935–36, **21**, 428–431.

[*Illustrations follow*]

DESCRIPTION OF PLATES

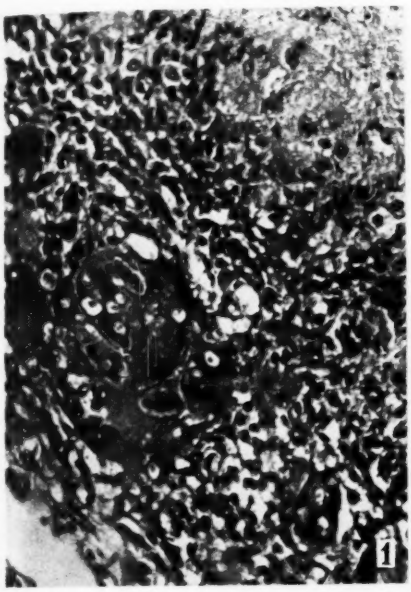
PLATE 179

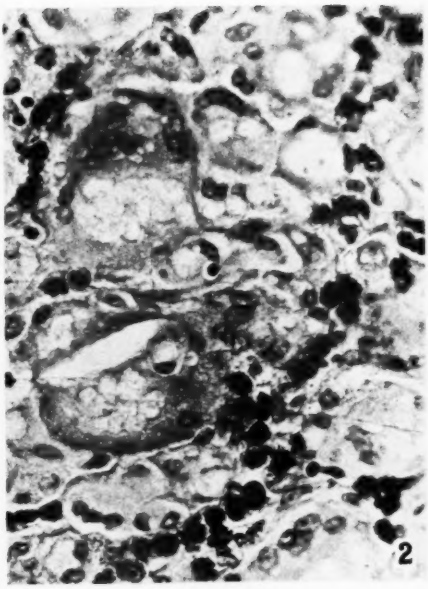
FIG. 1. Rabbit 902. Omentum on the 15th day after the intraperitoneal injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 400.

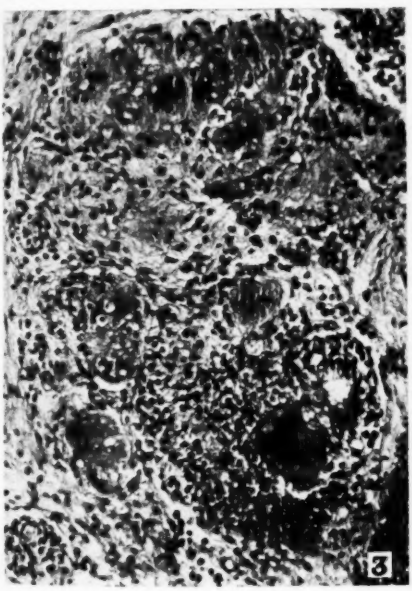
FIG. 2. Rabbit 909. Omentum on the 15th day after the intraperitoneal injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 500.

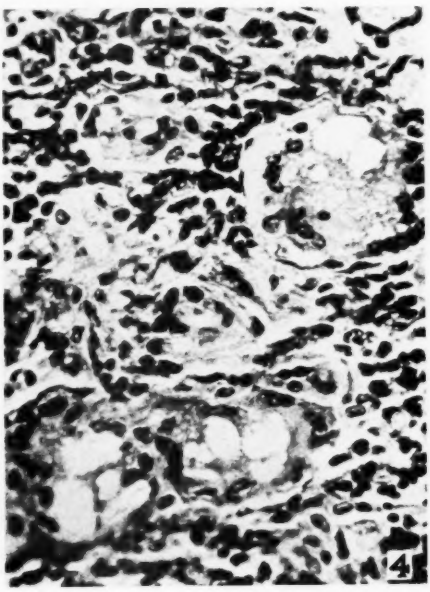
FIG. 3. Rabbit 894. Omentum on the 15th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 225.

FIG. 4. Rabbit 898. Omentum on the 15th day after the intraperitoneal injection of leprosinic acid. Hematoxylin and eosin stain. × 400.









Gerstl, Tennant, and Pelzman Cellular Reactions to Mycolic Acids

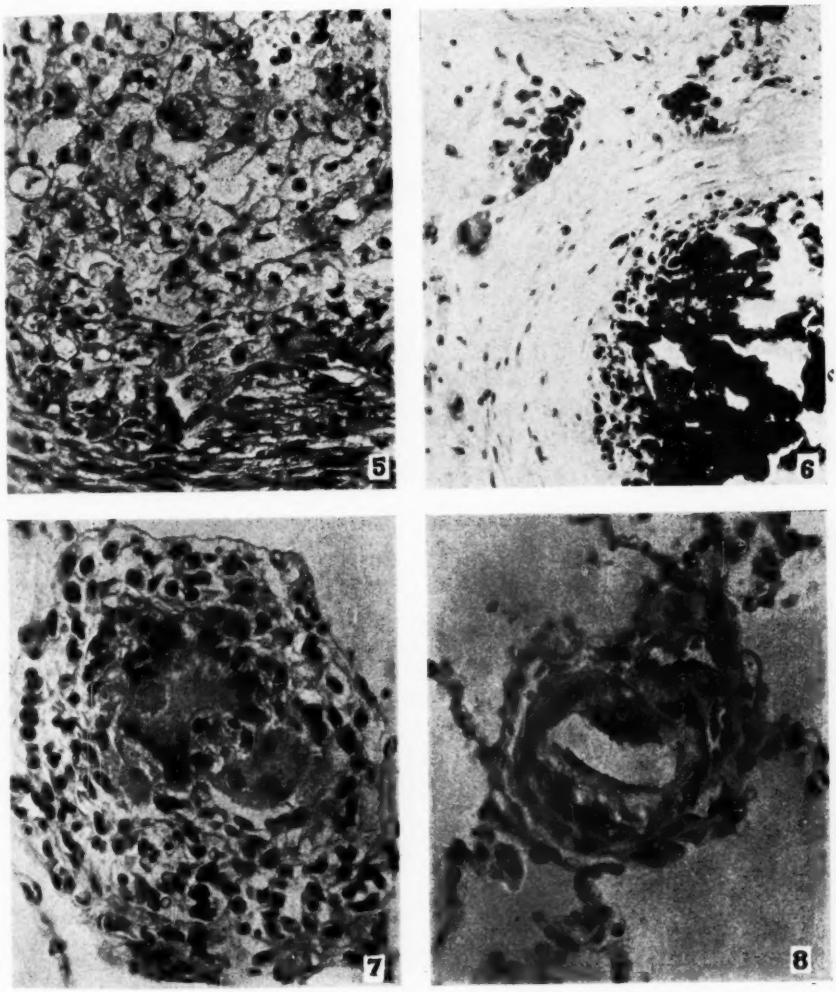
PLATE 180

FIG. 5. Rabbit 899. Omentum on the 45th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 285.

FIG. 6. Rabbit 899. Omentum on the 45th day after the intraperitoneal injection of bovine mycolic acid. Hematoxylin and eosin stain. × 235.

FIG. 7. Rabbit 63. Lung on the 5th day after the intravenous injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 325.

FIG. 8. Rabbit 61. Lung on the 5th day after the intravenous injection of leprosinic acid. Hematoxylin and eosin stain. × 325.



Gerstl, Tennant, and Pelzman Cellular Reactions to Mycolic Acids

PLATE 181

FIG. 9. Rabbit 66. Lung on the 5th day after intravenous injection of avian mycolic acid. Hematoxylin and eosin stain. × 700.

FIG. 10. Rabbit 13. Lung on the 15th day after the intravenous injection of a low-melting human mycolic acid. Hematoxylin and eosin stain. × 475.

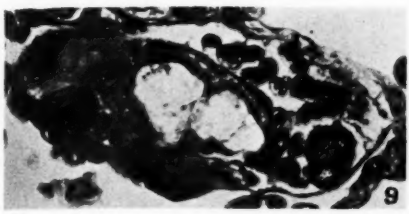
FIG. 11. Rabbit 16. Lung on the 15th day after the intravenous injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 325.

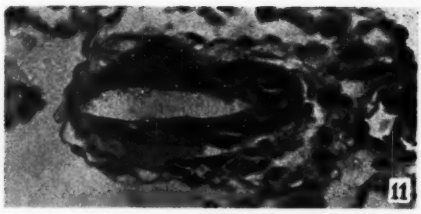
FIG. 12. Rabbit 30. Lung on the 15th day after the intravenous injection of leprosinic acid. Hematoxylin and eosin stain. × 325.

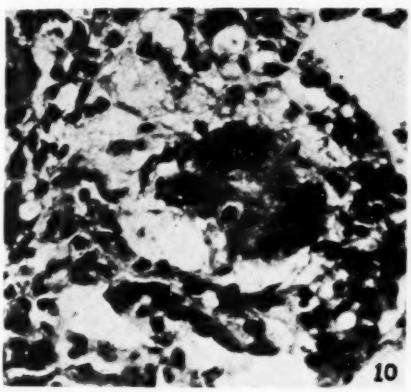
FIG. 13. Rabbit 22. Lung on the 45th day after the intravenous injection of a high-melting human mycolic acid. Hematoxylin and eosin stain. × 475.

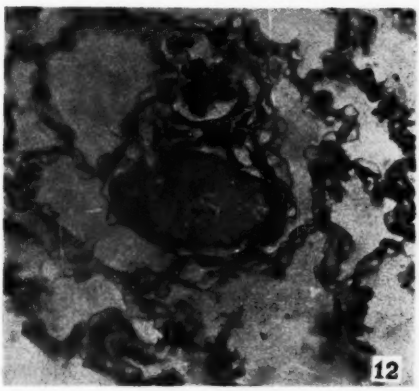
FIG. 14. Rabbit 29. Lung on the 45th day after the intravenous injection of bovine mycolic acid. Hematoxylin and eosin stain. × 485.

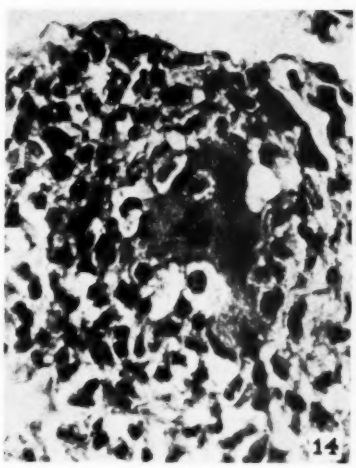
FIG. 15. Rabbit 18. Lung on the 45th day after the intravenous injection of avian mycolic acid. Hematoxylin and eosin stain. × 400.

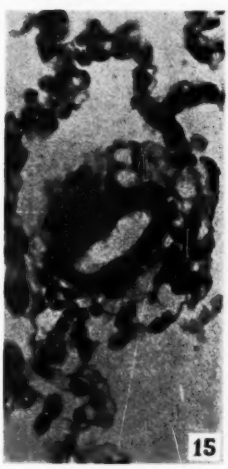












UNIV. OF MICH.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

XI. Thesaurosis and Atheromatosis Produced in Dogs by the Repeated Intravenous Injection of Solutions of Sodium Cellulose Glycollate *

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It was shown in previous investigations that colloidal aqueous solutions of methyl cellulose are effective substitutes for plasma and elicit, when introduced in highly excessive amounts over prolonged periods, symptoms of thesaurosis and atheromatosis (Hueper,[1, 2] Hueper and Ichniowski [3]). When a new water-soluble derivative of cellulose, the sodium salt of cellulose glycollic acid, became available, a toxicopathologic study of this substance appeared to be of interest.

Experimental Procedure

Sodium cellulose glycollate (Collocel S), which is manufactured by the Dow Chemical Co. in three grades of viscosity, is a cellulose ether with a carbomethoxyl ($-OCH_2COOH$) radical. As the degree of substitution is relatively low, there is a predominance of free hydroxyl groups. Collocel S of high viscosity is a white, odorless, granular powder readily soluble in cold and boiling water, giving a slightly turbid, grayish white solution of neutral reaction. After being filtered through Hyflo-Supercel † and filterpaper, a slight haziness remains. According to the statements of the manufacturer, Collocel is compatible with the majority of the acid dyes, but with very few of the basic dyes. Solutions of Collocel do not form foam upon shaking. A 0.25 per cent aqueous solution has a colloidal osmotic pressure of approximately 140 mm. of water.

A 0.25 per cent solution of the high viscosity grade of Collocel in 1 per cent sodium chloride solution was used in the experiments. It has a viscosity of 1.8 at 18°C. This solution was injected into the jugular veins of 5 dogs, 3 of which weighed 8 to 8.5 kg., 1 weighed 10.3 kg., and the fifth, 14.5 kg. Four of the dogs were from 1.5 to 3.5 years old, while the fifth was 6 years old. For the study of the immediate hematic reactions 4 dogs received a single dose of 40 cc. Blood was withdrawn 5, 15, 30, 60, 120, 240 minutes, and 24 hours after injection. The following blood constituents were determined: Amount of hemoglobin, number of erythrocytes and leukocytes, differential count, sedimentation rate, volume of packed blood cells, plasma viscosity. Additional daily injec-

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† Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N.Y.

tions of the same dose were made in 2 dogs after an interval of 7 days. After having been continued for 3 months, the daily dose was increased to 100 cc. for an additional period of 4 weeks, when the surviving dog was sacrificed after receiving a total of 4800 cc. of the solution containing 12 gm. of sodium cellulose glycollate. The second dog belonging to this set died after having received 7 injections of 100 cc. each.

The other 2 dogs used in the studies of the immediate reactions as well as a fifth dog received, beginning 24 hours after the first injection, daily intravenous administrations of the Collocel solution. The daily dose was 40 cc. for the first month, was increased to 75 cc. during the second month, and to 150 cc. during the third month. One of these 3 dogs was sacrificed after 16 days, the other 2 after 2.5 months, after having received a total of 6,485 cc. of the solution containing 16.25 gm. of sodium cellulose glycollate.

Hematic Reactions after a Single Intravenous Injection of Collocel

The erythrocytes increased from original values, fluctuating between 7.0 and 7.4 million, to values of 8.5 to 8.8 million 5 minutes after the injection of Collocel. This movement was followed by a gradual drop to approximately original levels during the following 2 hours when a second elevation of the erythrocytes was seen in the tests made at the 2 and 4 hour periods. The erythrocytes ranged then from 8.0 to 8.7 millions. Approximately original values prevailed again at the 24-hour test. The hemoglobin values underwent less appreciable changes and showed more often a tendency to decline than to rise. There occurred in all instances a moderate to marked leukopenia 5 minutes after injection, which lasted from 5 minutes to 3 hours in the different animals, but was followed in only one instance by a secondary leukocytosis. The differential counts revealed a mild shift toward the left coinciding with the leukopenic phase, but this development remained, in general, within normal limits. There were no appreciable or significant fluctuations in the viscosity of the plasma or in the sedimentation rate of the erythrocytes during the first 24 hours after injection. The platelets were extraordinarily large and of "parasite-like" appearance due to the frequent presence of flagella-like processes.

Hematic Reactions after Repeated Intravenous Injection of Collocel

The repeated intravenous injection of Collocel solutions was well tolerated. There were no temperature reactions, but immediately after each injection a transitory leukopenia developed. During the third and

fourth months the amount of hemoglobin gradually decreased to approximately 75 to 80 per cent of the original level. No significant abnormalities were found in the number of erythrocytes and leukocytes, nor in the differential count and the viscosity of the plasma. The sedimentation rate, on the other hand, increased progressively during the last 2 months, when it fluctuated between 30 and 40 mm. (Wintrobe-Landsberg tube).

Pathological Observations

The post-mortem examination of the 5 dogs did not reveal any appreciable macroscopic abnormalities of the internal organs. Particularly, the liver and spleen were not significantly enlarged nor did they deviate in consistency and color from normal organs.

Histologic studies were made of the lungs, heart, thyroid, parathyroid, liver, spleen, pancreas, intestine, adrenal, kidney, testis, aorta and its large branches. The aorta was cut for this purpose into 15 to 20 rings, while 2 or 3 rings each were cut from the carotid, brachial, and iliac arteries.

The dog which was sacrificed on the 16th day of the experiment had histologically normal organs with the exception of the presence of scanty amount of bluish homogeneous deposits found intracellularly and extracellularly in the splenic pulp in sections stained with hematoxylin and eosin.

The histologic findings in the other 4 dogs were essentially uniform. The ascending aorta showed occasional, small, localized proliferations of endothelial cells forming bundles running parallel to the longitudinal axis, and large fibroblastic-mononuclear edematous cushions containing numerous multinucleated giant cells. Foam cells filled with homogeneous matter, staining a moderately dark blue, were present in parts of these cushions and, in a few places, beneath the endothelial lining. In some segments of the thoracic aorta scattered small elevations of endothelial foam cells containing bluish matter as well as extracellular bluish material occurred beneath an endothelial lining showing cellular crowding. Such foci were observed at the orifices of branches, where they were found within, or extended into, the funnel-like first part of the branching vessel (Fig. 1). Areas exhibiting foam-cellular swelling of the endothelial lining were scattered here and there (Fig. 2). Similar foam cell lesions were encountered in several vasa vasorum located in the media or adventitia where foam cells were sometimes seen in the perivascular spaces of these vessels. In the lumen of one vas vasis a bluish mass was split up by numerous slender cells (Fig. 3). The inner media was often edematous, while fibro-hyaline foci were occasionally

seen in the outer media. Similar foam-cellular lesions occurred rarely in the large arterial branches of the aorta of only one animal. Atheromatous lesions in general were small and infrequent.

The Kupffer cells of the liver were balloon-like, swollen and proliferating, and contained a blue-stained homogeneous cytoplasm, while the nucleus was crescent-shaped and pushed against the cellular wall. The liver cells were intact. The pulp of the spleen contained many bluish staining foam cells as well as strands of a homogeneous extracellular bluish material. Mononuclear giant cells in a moderate number were present in the spleen of one dog (Fig. 5). The renal glomeruli contained scattered groups of swollen endothelial cells with a bluish content. Similar foam cells were occasionally seen in groups in the interstitial tissue (Fig. 4), particularly in the perivascular areas. Bluish staining casts were found in the tubular lumina of the medulla.

All other organs were without any significant changes.

Comment

The hematic and anatomic changes observed in dogs after the intravenous injection of solutions of sodium cellulose glycollate resemble in many respects those seen in dogs and rabbits after the intravenous introduction of colloidal solutions of other macromolecular carbohydrates such as methyl cellulose, polyvinyl alcohol, pectin, gum arabic (Hueper[2]), inasmuch as there appeared a transitory leukopenia, a decrease in hemoglobin, an increase in the sedimentation rate of the erythrocytes, a deposition of the injected matter in the reticulo-endothelial cells of the liver and spleen and in the glomerular endothelial cells and interstitial cells of the kidney, and the formation of atheromatous deposits in the aorta and its branches. It is remarkable, on the other hand, that these reactions remained within relatively moderate limits when compared with those seen in connection with the other above-mentioned substances, especially the methyl celluloses, if proper consideration is given to the total amount of colloidal material injected. The reason for this discrepancy must remain uncertain as no data are available on the molecular size of this compound and on the rôle which the glycollic acid part of the molecule might have played in its colloidal dispersion, in the colloidal stability of the plasma, and the permeability of the filtration membrane. The presence of bluish staining casts in the tubular lumina of the kidney indicates that the injected material is excreted through the kidney.

In contrast to methyl cellulose, sodium cellulose glycollate is not taken up by the liver cells, but only by the stellate cells. The atheromatous formation evidently occurs, as with other colloidal and macro-

molecular atheromatogenic agents, by the intracellular resorption of this material from the plasma into the endothelial cells and by its extracellular infiltration into the subendothelial spaces of the aorta and its large branches as well as of their vasa vasorum. The localization of these deposits in the region of the orifices of branches furnishes additional evidence supporting the contention that the normal turbulence of the blood, and vibratory lability of the plasma colloids in certain areas produced thereby, represent important factors controlling the distribution of atheromas in the vascular tree (Hueper [4]).

While the reported evidence is not unfavorable as to the suitability of solutions of sodium cellulose glycollate as a plasma substitute, blood pressure measurements made on anesthetized dogs injected with solutions of this substance showed that it exerts a definite depressor effect which obviates its use as a therapeutic agent in the treatment of shock.

Conclusions

Solutions of sodium cellulose glycollate, injected intravenously, elicit in dogs a transitory leukopenia and, upon repeated introduction, a decrease in hemoglobin and an increase in the sedimentation rate.

Sodium cellulose glycollate is stored in the Kupffer cells, the reticulum cells of the spleen, the endothelial cells of the renal glomeruli, and as atheromatous deposits in the aorta and its large branches.

The anatomical character and the distribution of the atheromatous lesions support the concept that in the genesis of these changes the macromolecular colloidal matter present in the plasma is taken up by the endothelial cells, which are transformed thereby into foam cells, and infiltrates extracellularly into the subendothelial spaces in which normally a turbulence of the blood exists and where thereby a vibratory lability of the plasma colloids is produced.

REFERENCES

1. Hueper, W. C. Reactions of the blood and organs of dogs after intravenous injections of solutions of methyl celluloses of graded molecular weights. *Am. J. Path.*, 1944, **20**, 737–772.
2. Hueper, W. C. Macromolecular substances as pathogenic agents. *Arch. Path.*, 1942, **33**, 267–290.
3. Hueper, W. C., and Ichniowski, C. T. The treatment of standardized and graded histamine shock in dogs with solutions of methyl cellulose and s-methylisothiourea sulfate. *J. Pharmacol. & Exper. Therap.*, 1943, **78**, 282–295.
4. Hueper, W. C. Arteriosclerosis. *Arch. Path.*, 1944, **38**, 162–181; 245–285; 350–364.

[*Illustrations follow*]

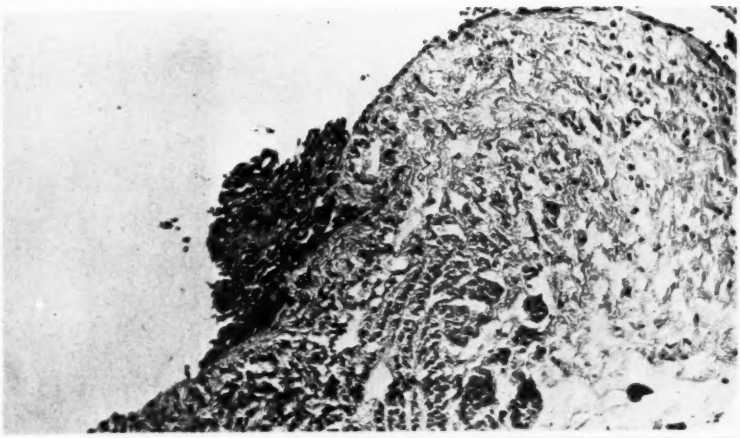
DESCRIPTION OF PLATES

PLATE 182

FIG. 1. Broad-based endothelial proliferation at the orifice of a small branch of the aorta. Hematoxylin and eosin stain. × 180.

FIG. 2. Endothelial foam cells coating the wall of the aorta. Hematoxylin and eosin stain. × 270.

FIG. 3. Foam-cellular proliferation in and around vasa vasorum of the aortic wall. Hematoxylin and eosin stain. × 270.



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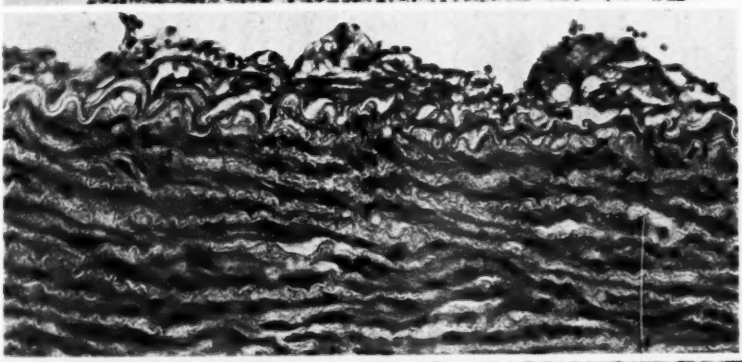

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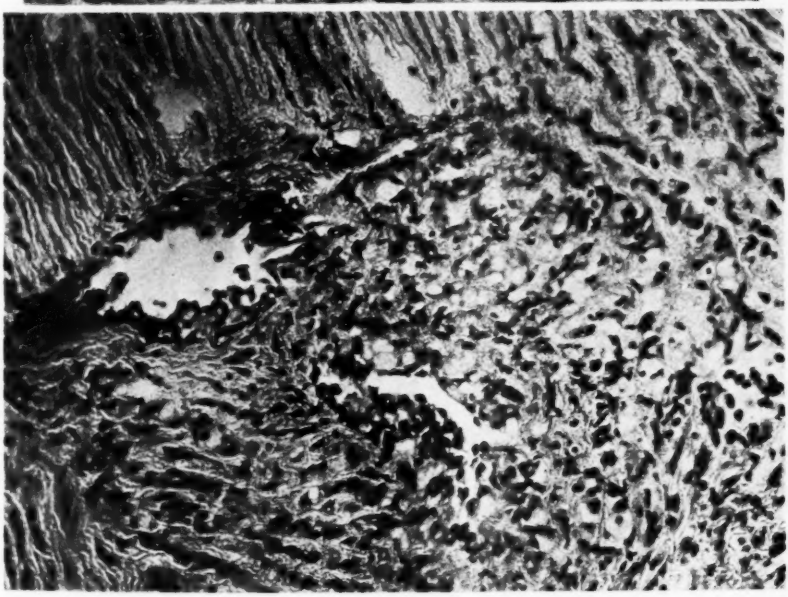

3

Hueper Sodium Cellulose Glycollate

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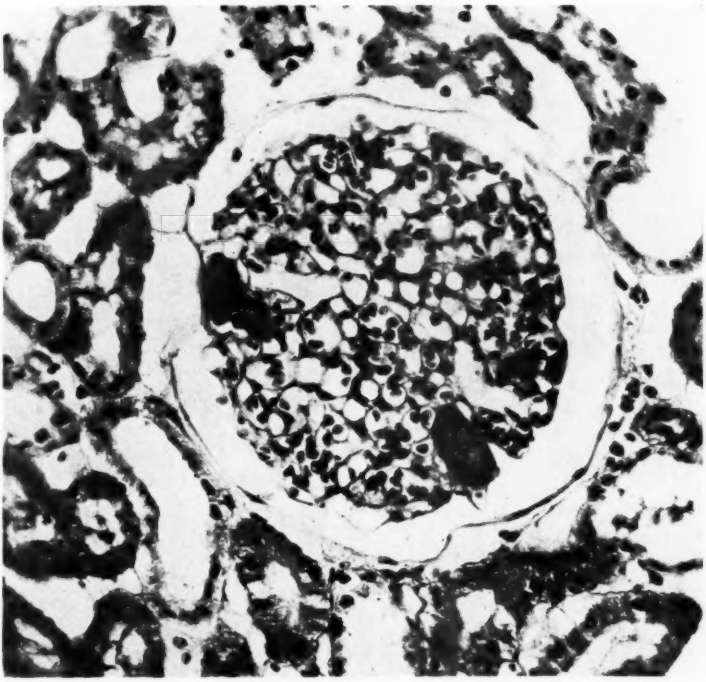
FIG. 4. Capillary loops of a glomerulus obliterated by swollen endothelial foam cells. Hematoxylin and eosin stain. × 360.

FIG. 5. Accumulations of reticulocytic foam cells in the pulp of the spleen. Hematoxylin and eosin stain. × 270.

4



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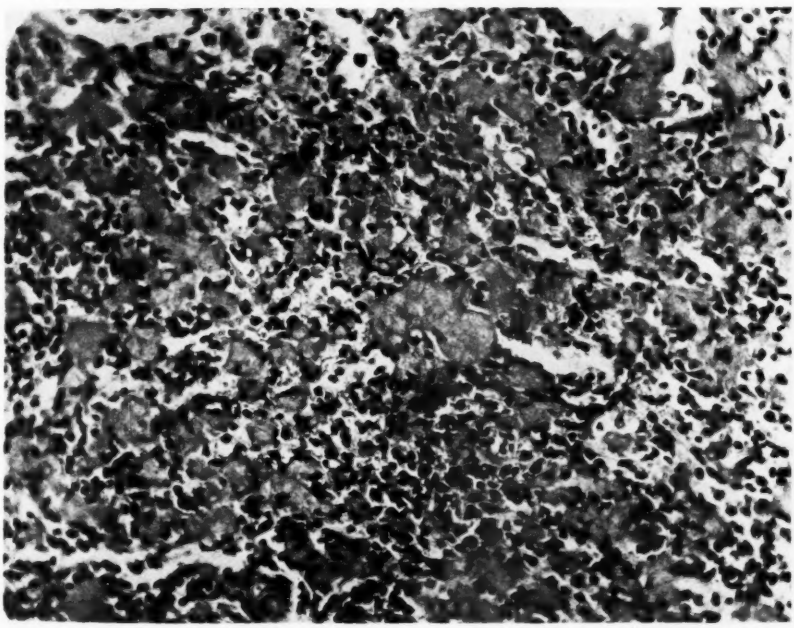

Hueper Sodium Cellulose Glycollate